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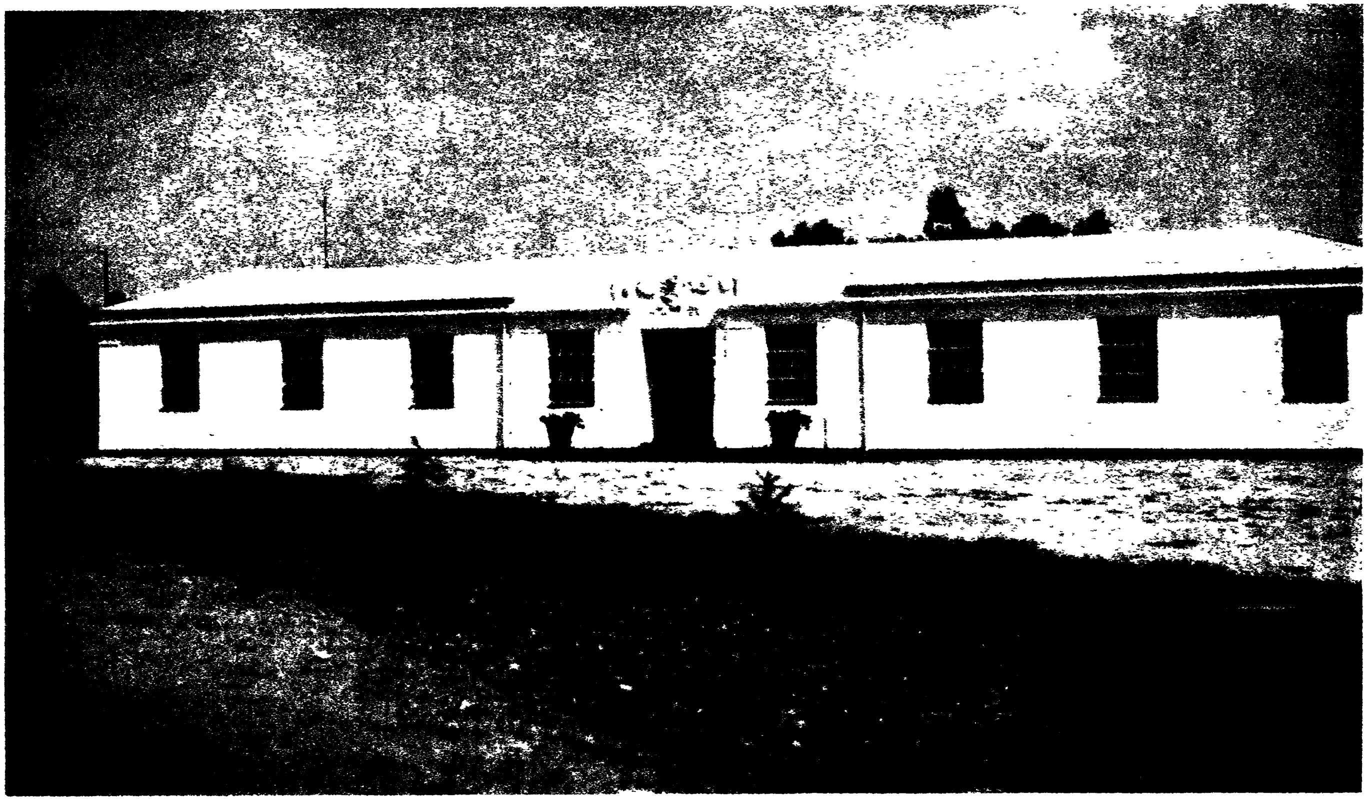
The Library Building, Onderstepoort—Erected 1932.



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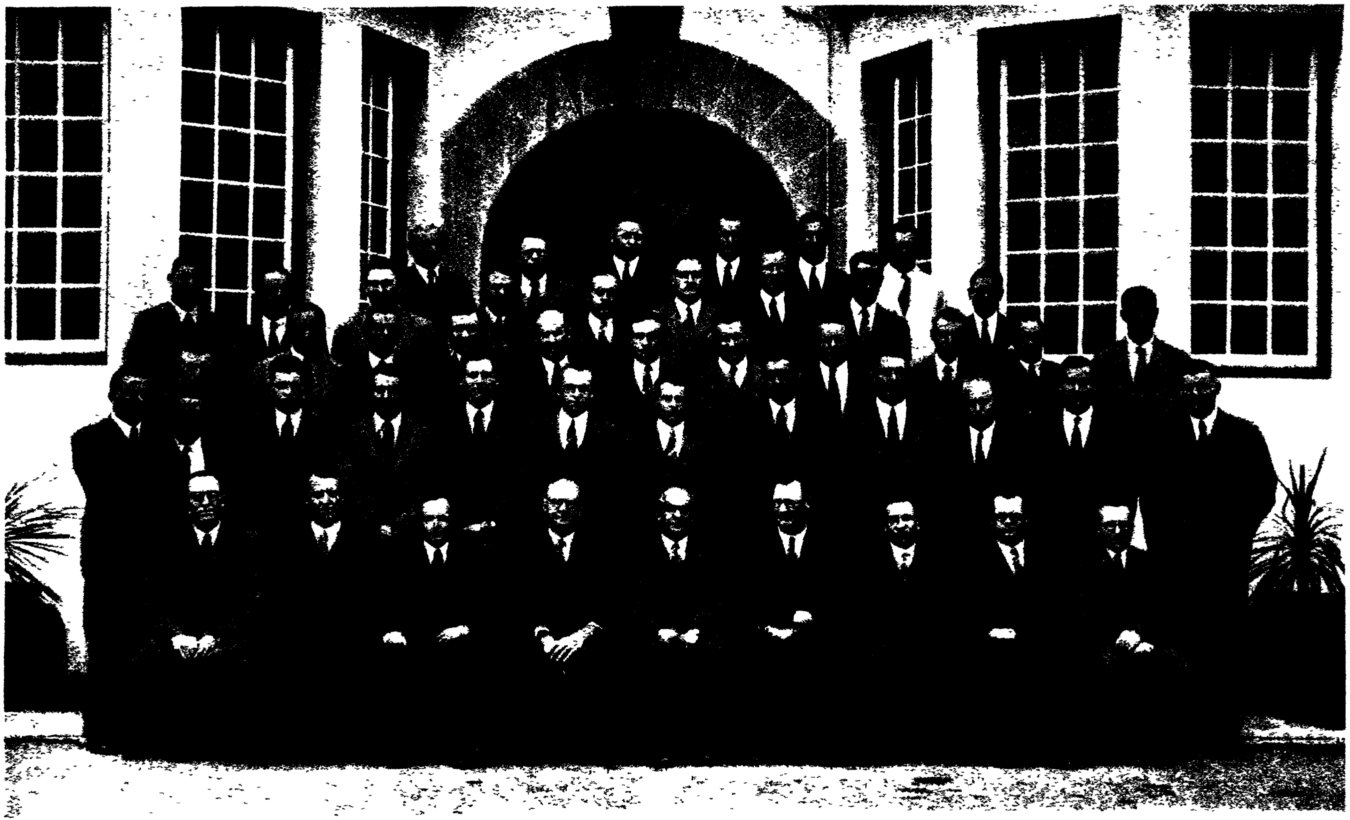

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Section I.

Protozoal Diseases.

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Surfen C. Therapy in *Trypanosoma congolense* Infection in Bovines and Ovines.

By S. W. J. VAN RENSBURG, Section of Medicine and Therapeutics, Onderstepoort.

THE only drugs that have up to the present been shown to be effective in the treatment of *T. congolense* infections are the antimony compounds.

Prior to the advent of Antimosan reliance was placed on Potassium Antimony Tartrate. This drug however is not very satisfactory because it has to be administered by the intravenous route, which factor militates against its general application under ranching conditions, especially when repeated injections are necessary.

Antimosan which is described as a complex of a trivalent antimony preparation with a pyrocatechin derivative was subsequently placed on the market, and this drug can be administered either subcutaneously or intravenously. Parkin (1930) concluded that as far as ease of administration is concerned Antimosan is immeasurably superior to Potassium Antimony Tartrate and that sterilization of bovines infected with *T. congolense* is obtained by the subcutaneous injection of 3 gms. Antimosan when given at four weekly intervals.

While there can be no objection to Antimosan as regards efficacy and local tolerability the fact that it has to be repeated three or four times is certainly a disadvantage. When therefore Surfen C. was made available with a claim that about 10 mg. per Kg. bodyweight given in a single dose either intramuscularly or subcutaneously will be a certain sterilizing dose in animals infected with *T. congolense*, it was hoped that a drug had been produced, which would be as efficient as the antimony preparations while possessing none of the disadvantages of the latter.

Surfen C. (Hochst 6678) is a Bayer preparation and is stated to be an amino-quinoline derivative, easily soluble in water. It is claimed that intramuscular injections are well tolerated without producing any general or local reaction.

It is claimed that "in calves the early treatment of a fresh congolense infection with even a single intramuscular injection of 5 mg. per Kg. effects a cure. In an infection existing for three months, three treatments each with 10 mg. Surfen C. per Kg. effected a complete cure.

"In experiments on sheep infected with *T. congolense* it was found that 10 mg. per Kg. Surfen C. cures every stage of the infection both in early and late treatment. . . . Fresh artificial infections of *T. congolense* in which treatment is initiated immediately after the appearance of trypanosomes in the blood can be completely cured with two subcutaneous injections of 5 mg. per Kg. given at an interval of one week. Also infections which had existed for one and three and a half months respectively were similarly treated with a single administration of double the above dose."

Surfen C. has been subjected to tests in various parts of the African Continent, but the reports regarding its efficacy and local tolerability are conflicting.

In Tanganyika, Hornby (1933) concludes that "The result which may be expected to follow a single intramuscular injection of Surfen C. is at least as good as the result to be expected from a course of five weekly injections of Antimosan or Tartar Emetic. . . . A local effect of slight swelling and tenderness, with or without slight lameness, and persisting for a few days was produced. . . . Post-mortem examination three to five days after injection showed no definite necrosis; merely hyperaemia and discolouration of fascia, Subcutaneous injections produced severe swellings lasting for weeks and are contra-indicated."

Mettam (1934) used the drug in the treatment of 19 head of cattle infected with *T. congolense* in Uganda, with the result that one animal died, but the rest "cleared up". He divided the dose (100 c.c.) into two parts and injected intramuscularly into either gluteal muscle and states that there is normally no local or systematic reaction. Mettam subsequently (1935) concluded that "this drug is the best trypanocidal agent available for the treatment of cattle trypanosomes. The antimonials are most efficacious against *T. congolense* and have but little action on *T. brucei*. Surfen C. on the other hand acts on all three cattle trypanosomes. Standardization of the preparation by the makers and the placing of it on the market as cheaply as possible is urgently needed".

After testing the drug on 14 horses in the Sudan, Bennett (1936) states that it is certainly capable of curing nearly all, if not all, cases of *T. congolense* in horses, but he regards the extreme variability of individual response to the drug as a serious drawback. A single injection frequently fails to sterilize and in one case as many as eight injections totalling 28·75 gms. had to be given before subinoculation showed the animal to have been sterilized. Troublesome local swellings are stated to be an added disadvantage.

His co-worker Evans (1936) concludes that in the case of cattle a single dose of Surfen C. offers as high a hope of effecting a complete cure as does a full course of Antimosan treatment.

In a memorandum submitted to the Conference for Co-ordination of Tsetse and Trypanosome Research in East Africa (1936), Danks and Daubney, of Kenya, give the results obtained by treating bovines with different issues of Surfen C.:—

(1) Two treated, both relapsed.

(2) Two treated, both sterilized.

(3) Five treated, four relapsed.

(4) Four treated, all regarded as cured.

Daubney is doubtful about the effect of the drug and believes that it has not yet been adequately standardized.

Stewart (1935) after treating animals with antimosan, tartar emetic and Surfen C. in the Gold Coast, expresses the opinion that treatment by Surfen C. does not appear to be nearly as effective as tartar emetic and other antimony preparations, though Surfen C. appears to be useful after antimony treatment to prevent relapses.

In Nigeria, Lester (1934) found that with sheep infected with *T. vivax* and *T. congolense* a dose of 10 mg. per Kg. proved to be more effective than a standard dose of tartar emetic, but three doses of Surfen C. given at weekly intervals did not prevent relapse. Six bovines were given three weekly doses of Surfen C. (10 mg. per Kg.) and twelve were given single doses of the drug. The experiments were complicated by an outbreak of rinderpest but from the point of view of a definite cure the results were not good as the majority of animals relapsed within a few weeks.

According to the 1935 report of the Veterinary Pathologist for Nigeria, six animals artificially infected with *T. vivax* which is the common trypanosome of cattle in Nigeria were treated with Surfen C. Two of these succumbed to trypanosomiasis, one continued to show *T. vivax* in smears, and though smears from the other three were negative, they continued to remain in a very emaciated condition. Large swellings, especially after injection of the salt solution, are mentioned as an objection to the use of Surfen C.

In a progress report on research on trypanosomiasis in Southern Rhodesia, Bevan (1937) states: "A number of drugs . . . have been tested as to their curative and preventive effects against various species of trypanosomes. The results in small animals have been disappointing. The so-called Surfen C., much vaunted as a remedy for *T. congolense* infection elsewhere, has not proved as successful against the local strains of trypanosome."

Method.

The animals used in the experiments under review were all artificially infected with various strains of *T. congolense* and were at no time exposed to natural infection.

Three different issues of the drug were used, and for the purpose of differentiating between these they are referred to as A, B and C.

Batch A consisted of the powder made up in ampoules each containing 2·5 gms., which was dissolved in 100 c.c. sterile distilled water.

Batch B was in liquid form as a 2·5 per cent. solution for intramuscular injection.

Batch C was a powder similar in appearance to A but stated to be less irritant. This was also injected in a 2·5 per cent. solution in sterile distilled water.

In view of the severe local and general reactions obtained by some of the other workers by the subcutaneous and intravenous injections of the drug, it was administered only by the intramuscular route in the present series of experiments. In bovines the injections were made into the gluteal and semi-tendinosus muscles and in sheep exclusively into the latter. No two injections were made at the same place.

Temperatures of the animals were taken twice daily, and blood smears daily excepting Sundays. In those cases in which smear examination was negative for some period following treatment subinoculations into cattle or sheep were carried out, and only when the latter yielded negative results were the animals in question pronounced sterilized.

The site of injection was examined at frequent intervals to observe the local reaction, and two bovines were subsequently killed for post-mortem examination of the injected areas. The injection of the first full dose given to bovine 9158 was made in one place, but subsequently all doses of 10 mg. per Kg. were divided into two equal parts and were injected at two different sites.

Local Tolerability.

In sheep the injections caused some degree of swelling accompanied by pain and lameness, which persisted for three or four days. In only one case (Sheep 45345 treated with batch C) did the swelling persist for about two months subsequent to the injection.

The local reaction was much more severe in bovines. Intramuscular injection of doses of 5 mg. per Kg. Surfen C. in a 2·5 per cent. solution produced in all cases painful swellings accompanied by lameness. The swellings persisted for some weeks or even months after injection and eventually subsided gradually and became hard persistent areas in the skin at the site of injection.

Bovines 5189 and 5198 were slaughtered on 18th November, 1936—over seven months after the last injection was made. Post-mortem examination showed localised schlerodermia with chronic myositis, subcutaneous melanotic areas at the sites of injection, and infiltration of the perivascular tissue and interfibrillar connective tissue with macrophages containing dark brown granules.

There appeared to be no variation in the intensity of the local reaction set up by the three batches of the drug.

The intramuscular injection of Surfen C. in the recommended doses was not followed by any general symptoms.

Discussion.

Full details concerning the experiments carried out to test the therapeutic value of Surfen C. in the treatment of *T. congolense* infection in bovines and ovines are given in Tables I and II. In the former it will be seen that the full recommended dose of 10 mg. per Kg. was given in the first instance to bovine 5198. This failed to sterilize, and in view of the very severe local reaction which it produced, an attempt was subsequently made to ascertain whether it was possible to obtain better results by administering smaller doses at varying intervals.

This animal was, therefore, given two doses of 2·5 mg. per Kg. each at an interval of twenty-four hours followed later by three similar doses. Thereafter two doses of 5 mg. per Kg. were given on two consecutive days and ultimately two injections, each consisting of 2·5 mg. per Kg. Surfen C. and 15 c.c. Antimosan, all of which failed.

The initial treatment for bovine 5189 consisted of two doses of 2·5 mg. per Kg. within twenty-four hours. After this, treatment at longer intervals was tried, five doses each of 5 mg. per Kg. being given at intervals of seven days.

Over a period of seven months bovine 5198 thus received 10 injections totalling 37·5 mg. per Kg. Surfen C. and 30 c.c. Antimosan without sterilizing, while 30 mg. per Kg. Surfen C. administered to 5189 in seven doses failed similarly.

Treatment at longer intervals, consisting of five doses Surfen C. each of 5 mg. per Kg. applied at weekly intervals to bovine 5526 failed to produce even a temporary disappearance of the parasites.

The full dose of 10 mg. per Kg. was reverted to in the treatment of No. 6026. The first two injections failed and sterilization was only effected by the third which, incidentally, was responsible for very marked swellings at the sites of injection.

In sheep initial treatment, consisting of two doses of 2 and 2·5 mg. per Kg. each given at twenty-four hours interval failed, while two injections of 5 mg. per Kg. given at a similar interval sterilized in one case (40933) and failed in another (41104).

As in the case of bovines, treatment at longer intervals with 5 mg. per Kg. given weekly failed (41104).

The full dose of 10 mg. per Kg. given all at once sterilized in one case (42768) but failed in two others (41029 and 45354).

It is apparent that the workers who have recorded good results from the use of Surfen C. are not all entirely convinced as to its efficacy. Mettam and Daubney both draw attention to the fact that the drug is not adequately standardized, and Danks and Daubney

are of opinion that later issues appear to be more effective. This view is not confirmed by the results of the present series of experiments, all three issues of the drug having failed equally badly.

The animals in these tests were stabled, and whether this lack of exercise could in any way have aggravated the local reactions cannot be definitely stated at present. These reactions, however, were of such a serious nature that even if the drug was 100 per cent. efficient therapeutically one would still be hesitant in recommending its general use by the intramuscular route.

The damage done by the drug to the subcutis and the musculature would seriously reduce the value of treated animals for slaughter purposes.

Conclusions.

(1) The intramuscular injection of Surfen C. in the treatment of *T. congolense* infection in bovines and ovines was found to be ineffective.

(2) The intramuscular injection of Surfen C. into bovines caused serious local reaction.

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TABLE I.

Surfen C. Therapy in T. congolense *Infection in Bovines.*

Bovine No.	Date Infected.	Date Trypanosomes Appeared.	Date Treated.	Dose in mg. per Kg.	Batch of Drug Used.	Smear Examination Negative. From.	Smear Examination Negative. To.	REMARKS.
5198	23.8.35	2. 9.35	12.9.35	10	A.	16. 9.35	11.10.35	Unsuccessful.
			31.10.35	2·5	A.	6.11.35	12.11.35	Unsuccessful.
			1.11.35	2·5	A.			
			20.11.35	2·5	A.			
			21.11.35	2·5	A.	23.11.35	5.12.35	Unsuccessful.
			22.11.35	2·5	A.			
			11.12.35	5	A.	17.12.35	31.12.35	Unsuccessful.
			12.12.35	5	A.			
			9. 3.36	2·5 — 15c.c Antimosan 12 per cent.	B.	—	—	Unsuccessful.
			7. 4.36	,,	B.	—	—	Unsuccessful.
5189	23. 8.35	31. 8.35	18.10.35	2·5	A.	19.10.35	13. 1.36	Unsuccessful.
			19.10.35	2·5	A.			
			9. 3.36	5	B.			
			16. 3.36	5	B.			
			23. 3.36	5	B.	9. 3.36	25. 5.36	Unsuccessful.
			30. 3.36	5	B.			
			7. 4.36	5	B.			
5526	28. 2.36	7. 3.36	11. 3.36	5	B.			
			18. 3.36	5	B.			
			25. 3.36	5	B.	—	—	Trypanosomes failed to disappear. Unsuccessful.
			1. 4.36	5	B.			
			9. 4.36	5	B.			
6026	12. 1.37	21. 1.37	30. 1.37	10	B.			Trypanosomes failed to disappear. Unsuccessful.
			19. 2.37	10	B.	26. 2.37	13. 3.37	Unsuccessful.
			24. 4.37	10	C.	26. 4.37		Sterilized.

TABLE II.

Surfen C Therapy in T. congolense *Infection in Sheep.*

Sheep No.	Date Infected.	Date Trypanosomes Appeared.	Date Treated.	Dose in mg. per Kg.	Batch of Drug Used.	Smear Examination Negative.		REMARKS.
						From.	To.	
43349	16.10.35	23.10.35	30.10.35	2	A.	1.11.35	15.11.35	Unsuccessful.
			31.10.35	2	A.			
			22.11.35	2·5	A.	29.11.35	9.12.35	Sheep killed on 24.12.35.
			23.11.35	2·5	A.			
40933	19.11.35	29.11.35	11.12.35	5	A.	14.12.35	—	Sterilized.
			12.12.35	5	A.			
41104	16.10.35	24.10.35	30.10.35	2·5	A.	2.11.35	26.11.35	Unsuccessful.
			31.10.35	2·5	A.			
			11.12.35	5	A.	13.12.35	14. 1.36	Unsuccessful.
			12.12.35	5	A.			
			9. 3.36	5	B.	12. 3.36	31. 3.36	Unsuccessful.
			16. 3.36	5	B.			
			23. 3.36	5	B.			
			30. 3.36	5	B.			
42768	19.11.35	20.12.35	9. 3.36	2·5 + 5c.c Antimosan	B.	10. 3.36	1. 4.36	Unsuccessful.
			2.11.36	10	B.	4.11.36	—	Sterilized.
41029	18. 8.36	29. 8.36	8. 9.36	10	B.	8. 9.36	26. 9.36	Unsuccessful.
			28.10.36	10	B.	—	—	Died on 29.10.36.
45354	22. 3.37	2. 4.37	24. 4.37	10 mg.	C.	26. 4.37	29. 4.37	Unsuccessful.

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Auto-Sterilization in Trypanosomiases.

By B. S. PARKIN, Section of Medicine and Therapeutics, Onderstepoort.

DURING the course of experiments on chemotherapy in Trypanosomiases due to *Trypanosoma congolense* and *Trypanosoma vivax*. it was observed that infected animals, some considerable time after treatment or even without treatment, made spontaneous recoveries. This article is for the purpose of placing the various cases on record.

The term auto-sterilization is used to denote the attainment of a completely sterile condition, as far as the particular parasite is concerned, in the absence of any treatment or subsequent to a relapse without further treatment being instituted. It is the spontaneous cure without chemo-therapeutical interference. Obviously the determination of the sterilized state is dependent on the technique utilized for determining whether the parasite is still present or not. Microscopic tissue examinations are somewhat unreliable for in many cases careful daily blood smear examinations over long periods have failed to reveal the parasites in known positive cases. The sub-inoculation of blood into rabbits, guinea-pigs and mice is also unreliable but that into animals of the same species has given very good results although failures by this method have also been met with. Re-inoculation ot the animal with the homologous parasite is of assistance and was used in a number of cases, Probably the best method is the complement fixation test but technical difficulties have interfered with its application in the trypanosomiases other than those caused by *T. brucei* and *T. equiperdum*.

The literature of recent years gives references to auto-sterilization in the trypanosomiases. There is a record by van den Branden (1926) of two patients who relapsed after treatment and who subsequently, without further treatment, showed no further parasites during a period of observation of two years. His examination comprised lumbar puncture and blood examination. Dyleff (1932) records his failure to detect, by triple centrifugation, trypanosomes in three Europeans who had previously been known carriers. Sen (1936) working with Surra in bovines obtained negative results when he sub-inoculated from untreated previously positive bovines into horses. Browning and Gulbransen (1935) record apparently spontaneous cure in mice infected with *T. brucei*. Manresa and Gonzalez

(1935) utilizing for the purposes of diagnosis microscopic examinations, animal sub-inoculations and complement fixation test show that a number of bovines infected with *T. evansi* made spontaneous recoveries. The period intervening from the time these animals were found to be positive to the time they gave negative results with the complement fixation test varied from 6 to 10 months. They say that revision is needed of the assumption so generally held that oxen once infected with *T. evansi* remain infected during their whole lifetime. Von Saceghem (1936) records spontaneous recoveries in calves inoculated with *T. congolense*. He tested by sub-inoculation but in his cases the recovered animals resisted subsequent re-infection.

Observations on Auto-sterilization.

Auto-sterilization has been observed in *T. vivax* and *T. congolense* but not in *T. brucei or T. equiperdum* infections. If it were not that the complement fixation test was available for the last two there would possibly have been a decision that auto-sterilization had also occurred in these for microscopic blood examination, sub-inoculations and re-infection all were at times inefficient for diagnostic purposes.

T. equiperdum Infection.

The diagnosis of *T. equiperdum* infection or dourine of horses is based almost entirely on the complement fixation test, the antigen used being *T. equiperdum* obtained from small laboratory animals. The sub-cutaneous inoculation of this parasite into natural dourine cases results in an acute reaction with the appearance of the parasites in blood smears, with a severe drop in the number of red cells and in the red cell volume, with exacerbations and remissions of temperatures and with a rapid loss of condition. The infected horses in other words have no premunition against the parasite which gives a positive C.F. Test when used as an antigen. If such re-infected animals are suitably treated there is a disappearance of the parasites from the blood which is permanent when judged by smear examination, a return to previous normality of red cell count, of red cell volume, of temperature and of condition but, notwithstanding, the horse remains positive to the C.F. Test carried out 16 months later, It it were not for the availability of this test, the horse might easily be regarded as sterilized. Only in one of the numerous smears examined has *T. equiperdum* been detected in smears and that was in a smear made from the mammary gland at autopsy in May 1935 by S. W. v. Rensburg of this section. If a susceptible horse be infected as above and treated, it becomes negative subsequently to the C.F. Test.

T. brucei Infection.

Horses infected with *T. brucei* and then treated may survive for long periods during which time microscopic examination may give negative results. Sub-inoculation into other horses may also at times give negative results but subsequent sub-inoculations with larger quantities of blood from such an animal have given positive results. The C.F. Test, *T. equiperdum* antigen being used, however always

gives a positive reaction in such cases. In donkeys daily negative smear examination over many months has been attained without the prior utilization of drugs. Such an infected donkey, not treated, has remained in good condition for the whole period of two and three quarter years. Both sub-inoculations into horses from this donkey and the C.F. Test give positive results. The cases of auto-sterilization in equines have thus only been apparent ones.

T. vivax INFECTION.

Five bovines infected with *T. vivax*, not treated were submitted to blood and gland smear examinations with positive results. Three of these bovines were killed as negatives as judged by smears between 18th and 44th months after injection. One died of a disease other than *T. vivax* infection at 2 years and the remaining one is still alive. From the last mentioned, (bovine 2611), eighteen sub-inoculations have been carried out only the first of which gave positive results. The first negative sub-inoculation was carried out 355 days after the date of infection.

T. congolense INFECTION OF SHEEP.

Auto-sterilization has been noted in a number of sheep. All the cases which have been observed were in infected sheep which were under uniform conditions of feeding and stabling. The details are recorded in Table 1.

Dis

The above details reveal that in all three cases sub-inoculation into sheep and bovines gave negative results, that in each case the test animals were tested for susceptibility by the inoculation of known infected blood and that in one case the susceptibility of the sheep itself was tested by the inoculation of known infected blood.

T. congolense INFECTION OF BOVINES.

Bovines infected with *T. congolense* when kept under conditions of good food and stabling pass often from the first acute stage into the chronic form of the disease. If such chronic cases be exposed to adverse conditions especially cold and rain, a change to the peracute form may occur as previously reported by the writer(1935). It was, therefore, decided to expose to similar adverse conditions, to determine whether relapses would occur, a number of bovines which were in the state of premunition, as judged by the persistence of the parasite in animals which were normal as far as their blood examination was concerned.

The arrangement of the experiment was (1) to run premune bovines in open kraals, where they would be supplied with adequate rations, day and night throughout the various seasons, and (2) to place them subsequently on natural grazing without supplementing the grazing with rations.

TABLE I.

Sheep No.	Date of Infection.	Last + Smear.	Subsequent — Smears.	Sub-inoculations.		Susceptibility Test.	
				Details.	Result.	Details.	Result
26786	7.8.31	26.6.32	—	18 11.32: Blood (10 c.c. S.C. and 40 c.c. I.V.) into sheep 34129	Negative...	7.3.33: Known infected blood (25 c.c. S.C. and 75 c.c I.V.) into sheep 34129	Positive.
				15.12 32: Blood (20 c.c. S.C. and 80 c.c. I.V. into sheep 34129	Negative...		
41867	26.4.35	16.6.36	243 smears up to to 23.4.37	20.11.36: Blood (20 c.c. S.C.) into bovine 6026	Negative...	12.1.37: Known infected sheep blood (20 c.c. S.C.) into bovine 6026	Positive.
				11.12.36: Blood (10 c.c. S.C. and 40 c.c. I.V.) into bovine 6026	Negative...		
37096	5.12.33	15.2.35	70· smears up to 23.8.35	2.8.35· Blood (20 c.c. S.C.) into bovine 5189	Negative...	23.8.35: Known infected sheep blood (20 c.c. S.C.) into sheep 37906	Positive.
						23.8.35: Known infected sheep blood (20 c.c. S.C.) into bovine 5189	Positive.

S.C. Subcutaneous inoculation.
I.V. Intravenous inoculation.

The exposure under the first heading was carried out at Onderstepoort concurrently with the exposure of chronic cases which, as a result, relapsed into peracute cases. All the premune bovines remained normal and grew satisfactorily. The winter was, that year, particularly severe and the animals were exposed to heavy winter rains and sleet. Smear examinations were regularly done and all the animals showed, on occasion, *T. congolense* in blood smears.

The exposure under the second heading was carried out on a neighbouring farm, " Kaalplaas ", in a camp which was heavily infested with ticks as dipping of the cattle had not been in operation for some years. In a few cases it was necessary to treat by hand the extensive ear damage produced by the ticks. No shelter was provided. In addition to the premune animals three bovines recently infected with *T. congolense* were added to the group as controls. All these controls died within a few weeks from *T. congolense* infection. In the premune bovines, no relapses were noted and they grew satisfactorily. A number of negative controls was also introduced. No cases of transmission occurred in these.

The number of premune bovines used was nine. The period of exposure under the first heading i.e. at Onderstepoort was 17 months (May 1930 to September 1931). Under the second heading it was 14 months (October 1931 to November 1932). The animals were submitted to a weekly examination which consisted of inspection for sickness and smear examination. During the first period no losses occurred but during the second period there was some mortality due to concurrent conditions. The last positive smear cannot be regarded as an indication of the commencement of sterilization for the reason that in all cases of premunition the trypanosomes are always difficult to detect. Consequently sub-inoculations into sheep and bovines in certain of the cases were undertaken at the close of the experiment to support the negative smear examination and the obvious healthy condition of the animals.

The experiment is detailed in Table II which records the last positive smear examination of the two periods, the fate of each bovine and the various sub-inoculations undertaken.

Discussion.

From the details available in the above table, it will be noted that the sub-inoculations into bovines and sheep of the blood of six of the bovines gave negative results notwithsanding that very large amounts of blood were used in all cases except one and that only a few months previously trypanosomes were found in blood smears. A few of the animals were sold on the open market. The average live weight of these animals was 1300 pounds. At the time of infection these animals averaged 590 pounds.

A later case of auto-sterilization in *T. congolense* infection of bovines enabled a more detailed observation to be carried out. This infected bovine 5189 was injected intravenously with a trypanocidal drug (Surfen C) on 5th and 24th January, 1934. Daily blood smear

TABLE II.

Number of Bovine.	At Onderstepoort. Last Positive Smear.	At Farm "Kaalplaas." Weekly Smear Examination.	Sub-inoculations. Details.	Sub-inoculations. Result.	Remarks.
2464	11/7/30	All negative..	10 c.c. blood into sheep 32342	Negative	Died of heart-water on 13/11/31
2468	1/8/30	All negative (additional smears made on last 3 days of life)	—		Died 3 days after calving on 14/11/31.
2471	11/7/30	All negative	100 c.c. blood into bovine 2782 on November 14th, 16th, 18th, 21st, and 23rd, 1932 and 10 c.c. into sheep 34559 on 7th Oct. and 16th Nov., 1932	Negative	
2714	24/2/30	All negative with exception of a positive smear on 9/4/32	Into bovine 3507 and sheep 34272 same quantities on same dates as in case of bovine 2471	Negative	
2994	16/6/30	All negative	Into bovine 2805 and sheep 34099 same quantities on same dates as in case of bovine 2471	Negative	
3525	23/3/31	All negative (smears on last 3 days of life)	-	—	Piroplasmosis on 14/11/31.
3637	16/1/31	All negative	—	—	Not Trypanosomiasis on 24/9/32.
3638	23/6/31	All negative with exception of a positive smear on 25/6/32	300 c.c. blood into bovine 3020 on 11/11/32 and 10 c.c. into sheep 34123 on 7/10/32	Negative	
3684	4/5/31	All negative with exception of a positive smear on 3/6/32	Into bovine 2765 and sheep 34201 same quantities on same dates as in case of bovine 2471	Negative	

examination gave 68 negative results up to 17th April, 1934, when sub-inoculation of blood (10 c.c. subcutaneously and 40 c.c. intravenously) into two sheep gave positive results. Up to 5th March, 1935, the examination of a further 262 smears gave negative results but

the sub-inoculation of blood (25 c.c. subcutaneously and 75 c.c. intravenously) on this date into two sheep now gave negative results. From the 12th to 24th June 1,000 c.c. of blood of this bovine was injected intravenously into a susceptible bovine 6026 with negative results. It was ultimately (on 23rd August, 1935) injected with 20 c.c. known infected blood subcutaneously and reacted with an incubation period of 8 days.

This case is a striking illustration of the inefficiency of blood smear examinations. For twelve weeks smear examination carried out on six days in the week gave negative results whereas the sub-inoculation gave positive results. Further smear examination up to the 46th week gave negative results. The sub-inoculation now also gave negative results. A later sub-inoculation of a large quantity of blood again gave negative results. If the smear examination had been used as the only criterion of sterilization, this bovine would have been classed as one sterilized by the injection of the Surfen C, an obviously incorrect conclusion.

It would appear that animals may attain, with or without the assistance of drugs, a state of premunition which is an equilibrium of the animal and the parasite with the former in a state of normality and that, in time, the animal body may obtain the upper hand with the result that the parasite is destroyed.

SUMMARY.

1. No cases of auto-sterilization have been observed in *T. brucei* or *T. equiperdum* infections of equines.

2. A number of cases of auto-sterilization is recorded in *T. vivax* infections of bovines and *T. congolense* infection of bovines and ovines.

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The Occurence of *Grahamella couchi* sp.n. in the Multimammate Mouse (*Mastomys coucha*) in South Africa.

By W. O. NEITZ, Section of Protozoology and Virus Diseases, Onderstepoort.

INTRODUCTION.

In 1903 Graham-Smith found intracellular parasites in the erythrocytes of approximately 10 per cent. of moles (*Talpa europea*) in England. Brumpt in 1911 named these parasites *Grahamella talpae*. Since the recognition of these organisms, no less than thirty species have been described from Europe, Africa, Asia and America, chiefly in the mammals belonging to the class Rodentia and Insectivora. The object of this paper is to record the occurrence of a species of *Grahamella* found in several multimammate mice caught on a farm adjoining the grounds of the Onderstepoort Veterinary Laboratory.

OBSERVATIONS.

During the month of September, 1936, several multimammate mice were examined. They were found to be infested with mites (*Laelaps muricola*) and lice (*Polyplax waterstoni*). Blood smears stained with Giemsa were prepared from seven mice. One of them was found to harbour *Trypanosoma lewisi*, and in the others no blood parasites could be demonstrated even after a prolonged search. In all the blood films Jolly bodies and polychromatic stained erythrocytes could be demonstrated. In order to ascertain whether the mice harboured parasites six were splenectomized. Blood smears were examined for a period of fourteen days and subsequently twice weekly for another six weeks. In four of the mice *Grahamella* appeared on the third day and in two on the fifth day after the operation. In the beginning the number of parasitized erythrocytes was extremely rare, but after a week 0·25 to 0·5 per cent. of the red-blood-cells were found to be infected. This level of infection was maintained for the rest of the period of observation. At the commencement of the infection the number of parasites per cell varied from 4-24, but after a month as many as 30-40 parasites were

frequently seen, and on some occasions as many as 72 organisms were observed. From these observations it would appear that although the number of parasitized cells was very low, the number of parasites in individual cells showed a noticeable increase.

Morphologically these parasites resemble those described in other species of animals. Measurements showed a variation of 0·5 to 1·0μ in length and 0·2μ in width.

Attempts to transmit these organisms to three normal rats, a splenectomized rat and two sheep gave negative results.

The question whether this parasite is distinct from any of the already described *Grahamella* is difficult to answer. The fact, however, that rats proved to be refractory is significant, since both the rat and the multimammate mouse belong to the same family Murinae. It is proposed to name this parasite provisionally *Grahamella couchi*.

SUMMARY.

The multimammate mice were found to be infested with lice and mites. One mouse was infected with *Trypanosoma lewisi*, and in six splenectomized mice one new species of *Grahamella* was found for which the name *Grahamella couchi* is proposed.

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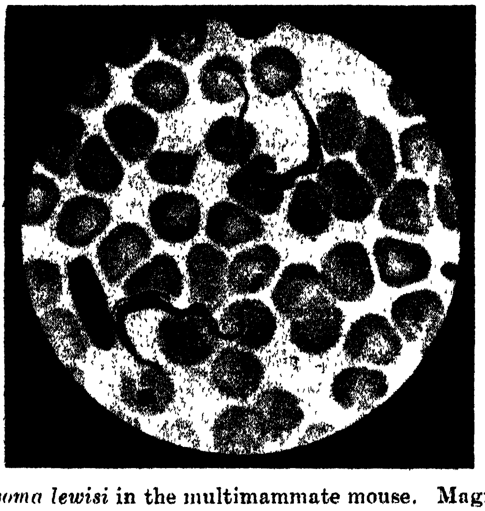

Fig. 1.—*Trypanosoma lewisi* in the multimammate mouse. Magnification 1,100×.

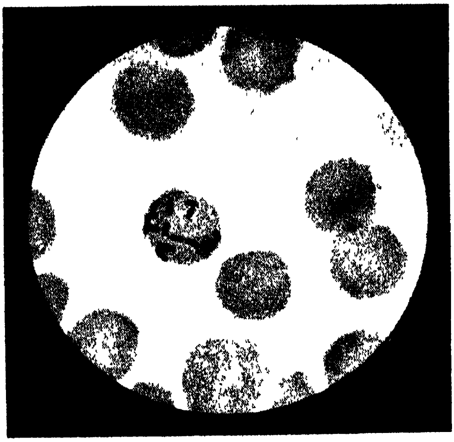

Fig. 2.—*Grahamella couchi.* Erythrocyte infected with 14 parasites. Magnification 1,500×.

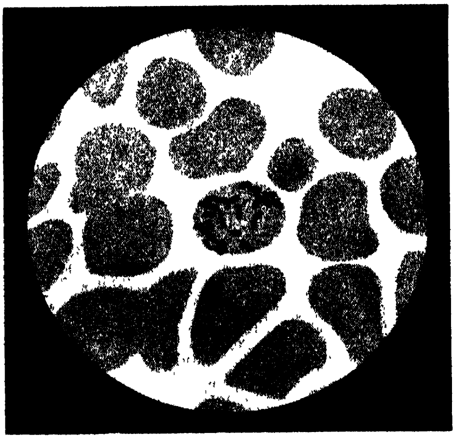

Fig. 3.—*Grahamella couchi.* Erythrocyte infected with 22 parasites. Magnification 1,500×.

"GRAHAMELLA COUCHI" IN MULTI-MAMMATE MOUSE.

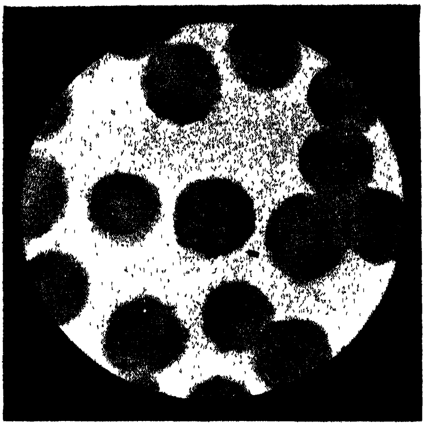

Fig. 4.—*Grahamella couchi.* Erythrocyte infected with 48 parasites. One free form is also present. Magnification 1,500×.

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The Destruction of *Piroplasma canis* by the Neutrophiles and Large Mononuclear Leucocytes.

By W. O. NEITZ, Section of Protozoology and Virus Diseases, Onderstepoort.

INTRODUCTION.

THE pathological changes observed in piroplasma infection in dogs depend on a number of factors such as the breed, age, general condition of the host, intensity of the infection and duration of the disease. The biliary fever parasites live and feed at the expense of the erythrocytes of which large numbers are destroyed. The anaemia that develops is not only due to a purely mechanical destruction of the cells, but possibly also due to a toxin that is liberated by the parasites. Toxins have not yet been demonstrated experimentally, but it has been observed that in chronic piroplasmosis where parasites are rare the anaemia may be marked. Such animals respond to treatment and the blood picture then returns to normal.

Another change observed in the blood is the leucocytosis. Nocard and Motas (1902) state that the leucocytosis is chiefly due to an increase in the large mononuclear and the polynuclear elements. The former phagocytose both erythrocytes and parasites. Phagocytosis occurs in the peripheral blood but is more marked in the spleen. Similar observations are recorded by Christophers (1907), Nuttall and Graham-Smith (1905) and Schuberg and Reichenow (1912).

Studies on the distribution of the parasites in the host were made by Kinoshita (1907) and Schuberg and Reichenow (1912). They found that most of the parasites are present in the capillaries of the skin and internal organs. A smear prepared from the first drop of blood that exudes from a small cut on the ear harbours many parasites, whereas already in the second drop great difficulty may be experienced in demonstrating the infection.

PERSONAL OBSERVATIONS.

In November, 1936, a Doberman Pinscher which had been suffering for several days from an acute attack of biliary fever was brought from a neighbouring farm to Onderstepoort for treatment.

The dog was injected with the recommended dose of acaprin, but six days later the owner reported that the dog died after showing convulsions.

A smear prepared at the time of treatment from the peripheral blood of the ear showed an interesting blood picture. The anaemic changes were advanced. Polychromasia, anisocytosis, Jolly bodies and normoblasts were a prominent feature. The differential count made from the peripheral blood was: Lymphocytes 22 per cent., large mononuclears 20 per cent., eosinophiles 1 per cent., basophiles 1 per cent., neutrophiles 56 per cent.

In the appended table the distribution of the parasites in the blood cells is given. A fair number of intracellular parasites was observed. On examining the leucocytes it was noticed that apart from the large mononuclears the neutrophiles were also actively engaged in destroying *P. canis*. The monocytes ingested both erythrocytes and parasites whereas the neutrophiles contained only the latter. The phagocytosed parasites showed various stages of degeneration. In some the cytoplasm was partially digested and in others only a disintegrated nucleus was visible.

The Distribution of Piroplasma canis *in the Various Blood Cells.*

Blood Cell.	Number counted.	Number containing parasites.	Remarks.
Erythrocyte......	1,000	56	Erythrocytes on an average contained 1 or 2 parasites. In some cells up to 8 parasites were seen. Extracellular forms were also present.
Large Mononuclears	1,000	148	The mononuclears harboured erythrocytes or parasites, but frequently both were present. 65 % of the mononuclears contained 1 parasite. 15 % of the mononuclears contained 2 parasites 11 % of the mononuclears contained 3 parasites 7 % of the mononuclears contained 4 parasites 1 % of the mononuclears contained 5 parasites 1 % of the mononuclears contained 8 parasites
Neutrophiles.....	1,000	90	Approximately 30% of the neutrophiles were band forms. Approximately 70% of the neutrophiles were segmented forms. 68% of the neutrophiles contained 1 parasite. 22% of the neutrophiles contained 2 parasites. 6% of the neutrophiles contained 3 parasites. 2% of the neutrophiles contained 4 parasites. 1% of the neutrophiles contained 5 parasites. 0·5% of the neutrophiles contained 6 parasites. 0·5% of the neutrophiles contained 7 parasites.

From this observation it would appear that the neutrophiles do not attack parasitized erythrocytes but only phagocytose the free parasites. Similar observations are recorded in malaria of human beings by Neumann and Mayer (1914) and Thomson and Robertson (1929), where the neutrophiles may harbour numerous parasites and pigment. Some authors (Hirschfeld and Sumi, 1925) state that the neutrophiles are also associated with erythrophagocytosis but De Kock and Quinlan (1926) were not able to demonstrate this phenomenon.

Summary.

A case of biliary fever in a dog is reported on where *P. canis* is phagocytosed by the large mononuclear leucocytes and the neutrophiles. In contradistinction to the activity of the mononuclears which ingest the parasites and their host cells the neutrophiles are only concerned with the destruction of the extracellular parasites.

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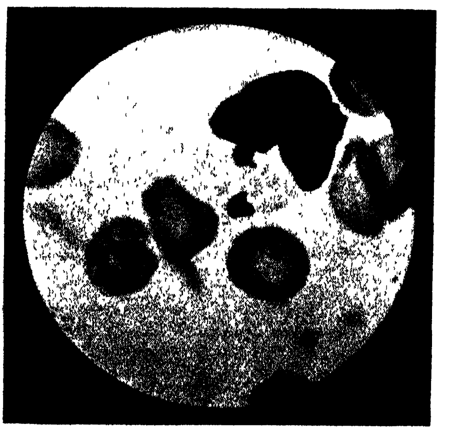

Fig. 1.—Neutrophile with 2 phagocytosed parasites (*P. canis*).

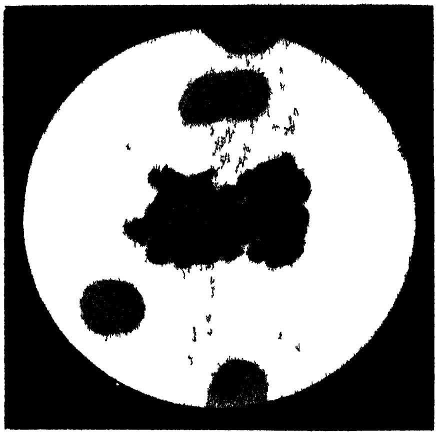

Fig 2—Neutrophile with 8 phagocytosed parasites (*P canis*)

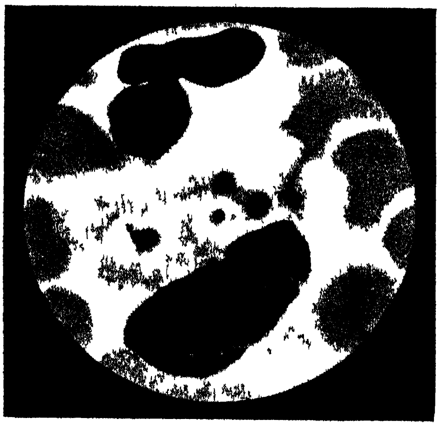

Fig 3—Large mononuclear leucocyte with 4 phagocytosed parasites In one parasite the cytoplasm has been completely destroyed and only the nucleus is visible (*P canis*)

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The Occurrence of *Nuttallia cynicti* Sp. nov. in the Yellow Mungoose *Cynictus penicillata* in South Africa.

By W. O. NEITZ, Section of Protozoology and Virus Diseases, Onderstepoort.

INTRODUCTION.

Blood parasites belonging to the family Babesidae have been described in four species of carnivora belonging to the family Viverridae. With the exception of Patton who records only the fact that he has seen piroplasms in the blood of a mungoose, the authors mentioned in the subjoined table have placed the parasites into the genus *Nuttalia*.

TABLE SHOWING THE VARIOUS SPECIES OF *Nuttalia* DESCRIBED IN THE FAMILY VIVERRIDAE.

Parasite.	Host. Zoological Name.	Host. Vernacular Name.	Country.	Author.	Year.	Measurements.
Nuttallia herpestedis	*Herpestes ichneumon*	Egyptian mungoose	Portugal....	Franca......	1908	Round forms 0·5–1·0μ. Pearshaped forms 1·5 × 1·8μ
Piroplasma species	*Herpestes edwardsi* *Herpestes mungo*	Mungoose.	India.......	Patton......	1910	—
Nuttallia civettae	*Viverra civetta*	Civet Cat.	Senegal.....	A. & M. Leger	1920	0·3, 0·4 and 1μ
Nuttallia legeri	*Herpestes calera*	Mungoose.	Africa......	Bedier.......	1924	1–1·5μ
Nuttallia cynicti	*Cynictis penicillata*	Yellow Mungoose	Orange Free State	Neitz........	1937	Round forms 1·8—2·2μ Ovoid forms 1·5 × 2μ Large round forms 3–6μ

Personal Observations.

Through the kindness of Dr. A. D. Thomas of this Institute the author received spleen smears prepared from several yellow mungoose (*Cynictis penicillata*) which were killed in the vicinity of Fauresmith in the Orange Free State in December, 1936. In two of the mungoose which were comparatively young blood parasites belonging to the genus *Nuttallia* could be demonstrated. In one animal the parasites were rare and in the other fairly frequent. The blood of the latter animal showed signs of anaemia, viz., anisocytosis, polychromasia and erythrophagocytosis of the parasitized cells.

Description of the Parasites.

Most of the parasites appeared as small circular bodies with a faintly blue staining cytoplasm and a dark red staining round nucleus situated at the periphery. These forms measured 1·8 to 2·2μ in diameter. Small ovoid forms measuring 1·5 by 2μ in size were also found. Large ring forms measuring from 3 to 6μ in diameter with an average of 4μ were not uncommon.

The parasitized cells usually contained one, two and four organisms and in some three, five, six, seven, eight or even sixteen parasites were seen.

Although the process of division into two was not observed it must be assumed that multiplication by this method did take place judging from the number of erythrocytes in which two parasites were found. Usually multiplication takes place by cruciform quadruple division characteristic of the genus *Nuttallia*. In the first stage the nucleus divides (see Fig. 3), the cytoplasm increases in size and the chromatin collects at the two opposite poles (see Fig. 4 and 5). The chromatin at the poles again divides (see Fig. 4), eventually giving rise to four parasites. These parasites may escape from the erythrocyte or may continue to multiply by the quadruple division thereby producing sixteen parasites as shown in Fig. 6.

Conclusions.

A blood parasite showing the characteristics of the genus *Nuttallia* has been described in the yellow mungoose. This species is considerably larger than those described by Franca, Leger and Bedier. The mode of transmission is not known.

It is provisionally proposed to name this parasite *Nuttallia cynicti*.

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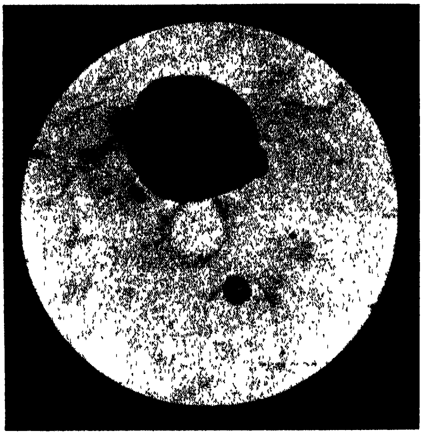


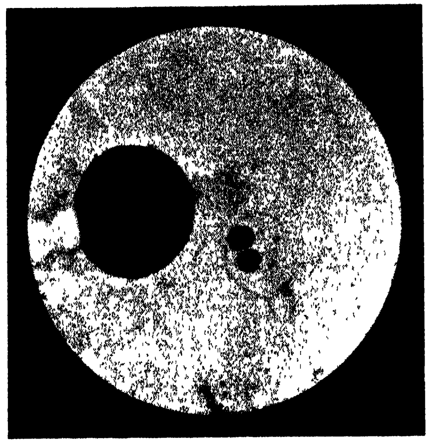
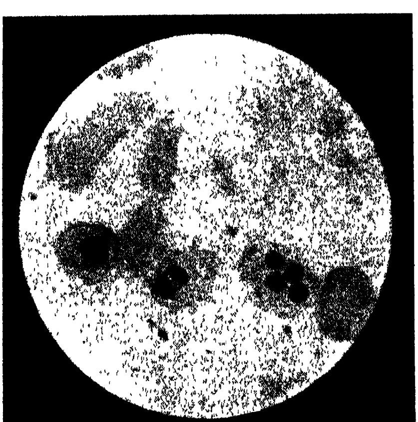


Fig. 1.—*Nuttallia cynıciti*. Magnification 1,500×.

Fig. 2.—Showing an erythrocyte infected with 2 parasites. Magnification 1,500×.



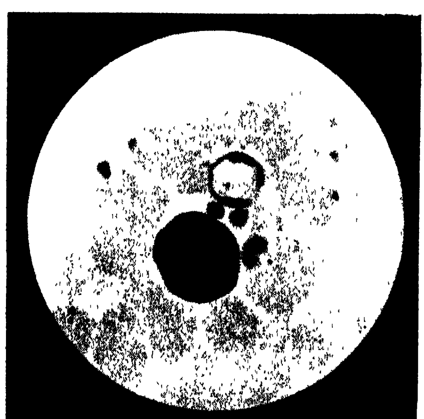


Fig. 3.—Showing erythrocytes infected with one, a dividing form and four parasites respectively. Magnification 1,500×.

Fig. 4.—Showing 4 small and one large parasite. Magnification 1,500×.

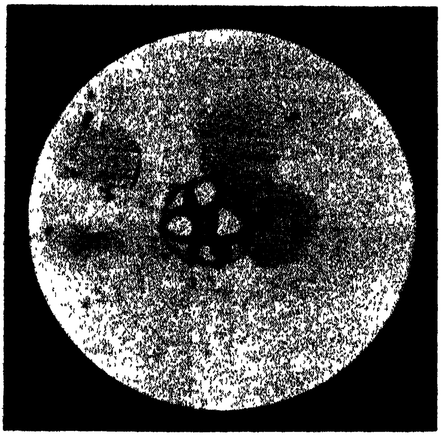


Fig. 5.—Showing 4 large parasites in an erythrocyte. Magnification 1,500×.

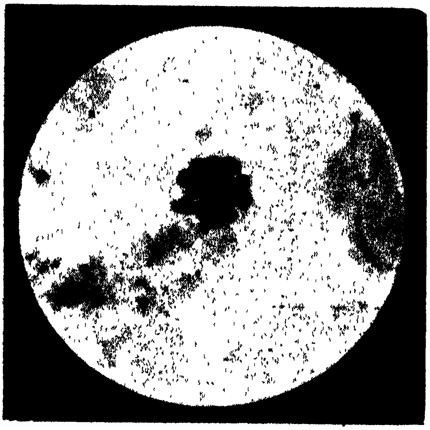


Fig. 6.—Showing 16 parasites in an erythrocyte. Magnification 1,500×.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 10, Number 1, January, 1938.

Printed in the Union of South Africa by the Government Printer, Pretoria.

The Appearance of *Bartonella muris* (M. Mayer 1921) in the Albino and Wild Rat after Splenectomy.

By W. O. NEITZ, Section of Protozoology and Virus Diseases, Onderstepoort.

INTRODUCTION.

IN 1921 Mayer described *Bartonella muris* in the blood of rats that had recovered from a severe attack of trypanosomiasis after the treatment with germanin. Further experiments by Mayer, Borchardt and Kikuth in 1925 showed that the anaemia and haemoglobinuria in splenectomized rats were produced by *B. muris* and not by an ultravisible virus as had been suggested by Lauda in 1925. The observations of Mayer have been confirmed by numerous workers from all over the world.

OBSERVATIONS.

The experiments to be described were undertaken in order to establish whether white mice and albino rats which are reared at the Onderstepoort Veterinary Laboratory and wild rats caught on the grounds of the institute harbour a latent infection of *Bartonella*. An examination of the rodents showed that they are infested with lice (*Polyplax serrata* and *P. spinulosa*) and on the wild rats fleas were also found.

Experiment 1.

Object.—To ascertain whether locally bred mice harbour any blood parasites.

Method.—Five white mice were splenectomized and blood smears stained with Giemsa were examined every alternate day.

Result.—The details of the observations are mentioned in Table 1. No parasites were found in any of the mice.

Experiment 2.

Object.—To infect the white mice with *Bartonella muris.*

Method.—Three of the mice that had remained free of parasites for a period of 28 days in experiment 1 were injected subcutaneously with 0·5 c.c. of blood from a splenectomized rat harbouring *B. muris.*

Result.—In one mouse *B. muris* appeared on the 8th day and in the other two on the 12th day after injection.

Numerous parasites were seen and a marked anaemia developed. One of the mice died on the 8th day after the appearance of parasites.

TABLE 1.

Splenectomy of White Mice.

No. of Mouse.	Date of splenectomy.	Smears examined for.	Result.	Date of Injection of *B. muris.*	Incub. period in Days.	Result.
1	22/9/36	8 days	Negative	—	—	—
2	22/9/36	14 days	Negative	—	-	—
3	22/9/36	28 days	Negative	21/10/36	8	*Bartonella muris* appeared on 29/10/36 in the blood. A severe anaemia and icterus developed but the mouse recovered.
4	22/9/36	28 days	Negative	21/10/36	12	*Bartonella muris* appeared on 2/11/36 in the blood. A severe anaemia and icterus developed but the mouse recovered.
5	22/9/36	28 days	Negative	21/10/36	12	*Bartonella muris* appeared on the 2/11/36. A severe anaemia and icterus developed and the mouse died 9/11/36.

Experiment 3.

Object.—To ascertain whether locally bred albino rats harbour a latent infection of *B. muris.*

Method.—Two rats were splenectomized and daily blood smears examined for a period of 14 days.

Result.—Both rats reacted to *B. muris* and recovered. See Table 2.

TABLE 2.

Splenectomy of Albino Rats.

No. of Rat.	Date of splenectomy.	Smears examined for.	Parasites appeared on.	Remarks.
1	24/9/36	14 days.	28/9/36	*Bartonella muris* appeared on the fourth day after splenectomy. A marked anaemia and icterus developed, but the rat recovered.
2	24/9/36	14 days.	28/9/36	*Bartonella muris* appeared on the fourth day after splenectomy. A marked anaemia and icterus developed but animal recovered. 27 days after splenectomy blood of this rat was injected into mouse 3, 4, and 5 mentioned in Table I.

Experiment 4.

Object.—To ascertain what parasites are harboured by wild rats caught at Onderstepoort.

Method.—Eight out of the ten rats were splenectomized and blood examined before and after splenectomy.

Result.—The details of the experiment are mentioned in the appended Table 3.

Four rats were infected with *T. lewisi*. Two rats died during the operation. Rat No. 5 did not react to *B. muris*, but died from *T. lewisi* infection six days after splenectomy. *B. muris* appeared on the 4th and 5th day in rats 6, 7, 8, 9 and 10.

Haemoglobin and bile pigments were found in the urine twenty-four hours after the appearance of parasites in rats 6, 7, 8 and 10. One rat recovered, two died and two were killed *in extremis*. The two control rats did not show any clinical symptoms.

Experiment 5.

Object.—To infect splenectomized sheep with *B. muris*.

Method.—The organs of rats 9 and 10 were emulsified and injected intravenously into two sheep 41496 and 41596.

Result.—Daily blood smears were examined for a period of 28 days, but on no occasion did *B. muris* appear.

TABLE 3.

Splenectomy of Wild Rats.

No. of Rat.	Examination of blood before splenectomy.	Date of splenectomy.	Bartonella observed on.	Haemoglobinuria observed on.	Remarks.
1	Negative...	20/4/37	—	—	Died from anaesthetic.
2	Negative...	29/4/37	—	—	Died from anaesthetic.
3	*T. lewisi* frequent	—	—	—	—
4	*T. lewisi* rare	—	—	—	—
5	*T. lewisi* very frequent	29/4/37	—	—	No *B. muris* appeared. *Tryp. lewisi* increased rapidly. The rat stopped feeding 4 days after the operation and died on the sixth day from the trypanosome infection.
6	*T. lewisi*	20/4/37	4th day..	5th day..	Died on the sixth day after the operation, showing marked anaemia and distension of the bladder with haemoglobin and bile stained urine. There was no perceptible increase of *T. lewisi*. *B. muris* appeared in extremely great numbers.
7	Negative...	20/4/37	4th day..	5th day..	Died on the seventh day after the operation showing marked anaemia, generalized icterus and distention of the bladder with haemoglobin and bile stained urine. *B. muris* appeared in extremely great numbers.
8	Negative...	29/4/37	5th day...	6th day.	*B. muris* appeared in extremely great numbers. Haemoglobinuria was noticed for two days. Rat recovered.
9	Negative...	29/4/37	5th day..	—	*Bartonella* frequent. Killed on the sixth day and organ emulsions were injected into 2 splenectomized sheep 41496 and 41596.
10	Negative...	29/4/37	4th day..	5th day..	*Bartonella* appeared in large numbers. Haemoglobinuria was noticed for one day. Rat was killed on the sixth day and showed generalized icterus, marked anaemia and bile pigments in the urine. Organ emulsions were injected into two splenectomized sheep 41496 and 41596.

Conclusions.

1. Five of the locally-bred mice did not harbour blood parasites although they were infested with lice. The mice were found to be susceptible to *B. muris*.

2. Splenectomy activated a latent infection of *B. muris* in two albino rats.

3. Four out of the ten wild rats were infected with *T. lewisi*.

4. Five out of the six splenectomized wild rats reacted severely to *B. muris*. One rat recovered, two died and two more were killed *in extremis*. The splenectomized rat that did not react to *B. muris* died from *T. lewisi* infection.

5. The two splenectomized sheep which were injected with *B. muris* were found to be refractory.

LITERATURE.

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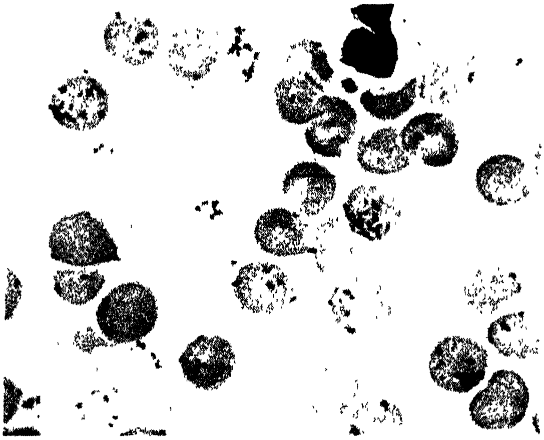

Fig. 1. - *Bartonella muris* in the blood of the albino rat, *Rattus rattus*. Magnification 1,100×.

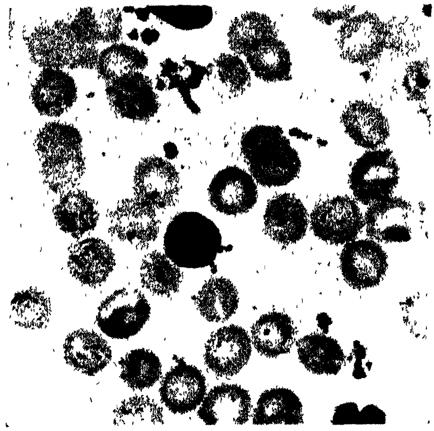

Fig. 2. *Bartonella muris* in the blood of the wild rat, *Rattus rattus* Magnification 1,100×.

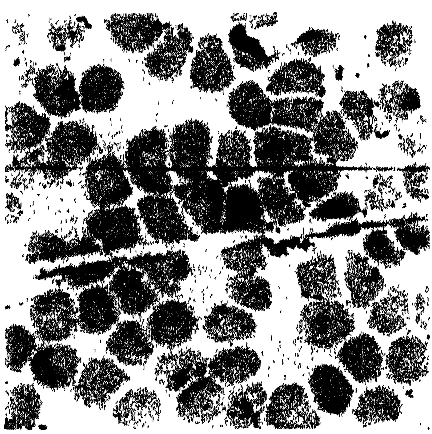

Fig. 3.—*Bartonella muris* in the blood of the white mouse, *Mus musculus*. Magnification 1,100×.

Section II.

Parasitology.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 10, Number 1, January, 1938.

Printed in the Union of South Africa by the Government Printer, Pretoria.

A Monograph of the Helminth Parasites of the Elephant.*

BY O. P. VAN DER WESTHUYSEN, M.Sc. (UNIV. OF S.A.).

INTRODUCTION.

SINCE the literature on the helminth parasites of the elephant is extremely scattered and contained in various periodicals, the object of this paper was to attempt to compose a synopsis of all the hitherto known helminth parasites of the Indian and African elephant.

The synopsis is not merely a compilation, but includes examination of material representing a large proportion of the parasitic genera. This detailed study showed that it was necessary to augment description of certain structures and also to amend the diagnosis of a few of the genera.

The material placed at my disposal for this investigation, has been contributed by Dr. H. O. Mönnig (Veterinary Research Officer at Onderstepoort), to whom I am also indebted for his suggestion as to the nature of the undertaking.

The specimens were collected from four circus elephants of the Indian species, all of which died as a result of the severity of their nematode infections. I also had the opportunity of examining some species from the African elephant, which Dr. Mönnig was so kind as to send me.

The nematode collection presented a very interesting study, and after it had been sorted out, proved to contain specimens of practically all the species so far described from the Indian elephant, with the addition of two new species belonging to the genera, *Choniangium* and *Grammocephalus*, respectively. The super-family STRONGYLOIDEA, is extremely well represented in the elephant. The family STRONGYLIDAE is represented by 6 genera including 35 species, of which 25 species are parasitic in the African elephant, and 12 in

* A portion of the nematode section of this paper was sumbitted as a thesis, presented in partial fulfilment of the requirements for the degree of Master of Science of the University of South Africa.

the Indian elephant. The family ANCYLOSTOMIDAE is represented by 3 genera including 7 species, of which 3 are parasitic in the African elephant, and 4 in the Indian. The family SYNGAMIDAE however, is represented by only one genus including one species from the Indian elephant. Of the 10 genera the following are found in the Indian elephant only: *Equinubria*, *Decrusia*, *Bathmostomum* and *Choniangium*.

It is interesting to note that the nematode parasites of the Indian and African elephant show a fairly strict host specificity, in so far that up to the present no one species has been found which is common to both hosts. In connection with this it is important to note that in the collection of nematodes worked through, a species belonging to the genus *Grammocephalus*, was found, which combines more or less the features presented by the two previously described species, one of which parasitises the Indian elephant, and the other the African elephant. This species, however, is fully discussed in this paper.

The STRONGYLIDS of the elephant are all parasitic in the alimentary tract, except the genus *Grammocephalus*, the adults of which inhabit the bile ducts, and in the case of one species of *Grammocephalus*, immature and larval forms were taken from nodules removed from the lining of the large intestine. This suggests that in the development of its life history, the larvae of *Grammocephalus*, which are probably introduced into the host with food, in course of its development penetrate the wall of the intestine from where, in course of time they probably re-enter the lumen of the intestine and then migrate directly up the bile duct. On account of the large size of the larval forms encountered in the intestinal nodules, it is impossible to conclude that these larvae might also reach the liver, and finally the bile duct, via the blood circulation.

A few larvae belonging to the genus *Murshidia*, were also found among the specimens collected from the lumen of the small intestine. *Murshidia*-larvae probably do not migrate, but develop in the intestine which is also the habitat of the adult forms.

A thorough investigation of the species at my disposal revealed certain structural characters which had been overlooked by previous workers.

All species of *Murshidia* encountered among the material, show the presence or the indication of a second coronal leaf-crown; this structure however, is not mentioned by Khalil, Lane, Witenberg, nor by the other investigators who either revised species of *Murshidia* already described, or added more species to the genus. It is however, indicated by Witenberg, 1925, in his figures of *M. murshida, M. falcifera,* and *P. neveu-lemairei*, and also by Neveu-Lemaire, 1928, in his figures of *M. brevicauda* and *P. omoensis*. Neveu-Lemaire actually describes a second coronal structure for *P. omoensis* from the African rhinoceros, and although he states that this species is indistinguishable from *P. omoensis* from the African elephant, he does not figure nor describe a second corona for the latter species. This second leafcrown is composed of numerous, very short elements, and has its origin at the anterior margin of the buccal capsule. It

must, however, be admitted that this structure is not equally distinct in all the species, and its presence is sometimes masked by the more pronounced leaflets of the first leafcrown. However, in the species of Murshidia in my possession, the coronal structure is extremely well marked, and in the large number of specimens examined, it is a very constant feature. In my opinion this is sufficient evidence to assume the presence of a second corona in all species of *Murshidia*, and this, I think, future investigations will show to be the case.

Also in the species of *Decrusia*, attention is directed to the presence of a poorly developed but very definite second (internal) coronal leafcrown.

In all members of the MURSHIDIINAE examined, the first (external) corona always has much longer and better defined leaflets. In *S. sipunculiformis* however, the external leafcrown is composed of small, short and stout elements.

Examination of the buccal capsules in many species, under very high powers, also revealed the presence of numerous small teethlike structures or tubercles at the base of the capsule. These structures, although of no great diagnostic significance, were also overlooked by previous workers. These were found present in all species of *Murshidia* examined, and also in *Amira piléata* and *Equinubria sipunculiformis*.

It is also interesting to note that many species, even from the same individual host, are subject to extreme variations. This is particularly the case among species of *Murshidia*, especially in regard to bursal rays in the male and the tail endings in the female.

In preparing this paper, I have so far as is possible relied for my illustrations and descriptions on personal observation, but I have of necessity also copied from the work of others. Their drawings have been acknowledged in the usual manner.

I wish to take this opportunity to acknowledge my thanks to Dr. G. Theiler, under whose supervision the major part of this investigation was carried out, and to whom I am very grateful for her useful criticism.

Also my grateful thanks to Dr. H. O. Mönnig for the use of his literature and of his camera-lucida drawings, as also to Dr. R. J. Ortlepp (Research Officer at Onderstepoort) for his valuable suggestions, and for so kindly placing several very useful references at my disposal.

TECHNIQUE.

The Nematode and Amphistome material was fixed in 70 per cent. alcohol. For study, however, the Nematode specimens were cleared in lactophenol.

The Amphistomes studied were stained with acid alum carmine, and serial sections, both transverse and sagittal, were made of some specimens, and other stained specimens were studied *en toto*.

CLASSIFICATION OF NEMATODES PARASITIC IN THE ELEPHANT.

Super-family.	Family.	Sub-family.	Genus.	Species.	Host.
I. Strongyloidea	1. Strongylidae	A. Murshidiinae	Murshidia	*M. murshida*	Ind. Eleph.
				M. falcifera	"
				M. indica	"
				M. neveu-lemairei	"
				M. linstowi	Afr. Eleph.
				M. hadia	"
				M. longicaudata	"
				M. brachyscelis	"
				M. africana	"
				M. anisa	"
				M. dawoodi	"
				M. omoensis	"
				M. brevicapsulatus	"
				M. memphisia	"
				M. aziza	"
				M. loxodontae	"
				M. soudanensis	"
				M. brevicaudata	"
				M. lanei	Ind. Eleph.
		B. Trichonominae	Quilonia	*Q. renniei*	"
				Q. travancra	"
				Q. apiensis	Afr. Eleph.
				Q. africana	"
				Q. uganda	"
				Q. brevicauda	"
				Q. ethiopica	"
				Q. khalili	"
				Q. loxodontae	"
				Q. magna	"
		C. Amirinae	Amira	*A. pileata*	Ind. Eleph.
				A. sameera	Afr. Eleph.
		D. Strongylinae	Choniangium	*C. epistomum*	Ind. Eleph.
				C. magnostomum	"
			Equinubria	*E. sipunculiformis*	"
			Decrusia	*D. additictia*	"
	2. Ancylostomidae	Necatorinae	Bunostomum	*B. foliatum*	"
				B. brevispiculum	Afr. Eleph.
				B. hamatum	"
			Bathmostomum	*B. sangeri*	Ind. Eleph.
			Grammocephalus	*G. clathratus*	Afr. Eleph.
				G. varedatus	Ind. Eleph.
				G. hybridatus	"
	3. Syngamidae		Syngamus	*S. indicus*	"
II. Oxyuroidea	Atractidae	Atractinae	Leiperenia	*L. leiperi*	Afr. Eleph.
				L. galebi	Ind. Eleph.
III. Ascaroidea	Ascaridae	Ascarinae	Toxocara	*T. longoptera*	Ind. Eleph.
IV. Spiruroidea	Acuaridae	Acuariinae	Parabronema	*P. indicum*	"
				P. smithi	"
				P. africanum	Afr. Eleph.
				P. rhodesiense	"

CLASS NEMATODA.

SUPERFAMILY STRONGYLOIDEA. Weinland, 1858; Hall, 1916.

FAMILY I, STRONGYLIDAE, Baird, 1853.

Sub-family I, Murshidiinae, Witenberg, 1925.

Diagnosis: Strongylidae, of medium size with a straight head and a mouth opening which is oval in cross-section. The mouth-capsule has the shape of an oval ring, of which the dorso-ventral axis is longer than the transverse. The cuticular layer which paves the mouth-capsule splits (at different levels according to different species) into narrow leaflets which form one row showing elongation of the leaflets laterally and shortening dorsally and ventrally. The base of the mouth-cavity leads to a funnel-shaped throat, which may or may not be provided with toothlike processes. Four submedian head-papillae are generally well developed, the lateral ones are on the contrary, generally very small.

The vulva is situated near the posterior extremity of the body. The female genital ducts show parallel uteri running cephalad, two ovejectors and two vaginal ducts which run together forming a short common portion terminally at the vulva (Fig. 1).

In the male there are two similar spicules and an accessory piece.

Genus Murshidia, Lane, 1914. (Amended.)

Syn. Pteridopharynx, Lane, 1921.

Memphisia, Khalil, 1922.

Henryella, Neveu-Lemaire, 1924.

Pterygopharynx, Witenberg, 1925.

Fairly slender worms tapering towards the head-end. Mouth directed straight forwards; mouth-collar is more prominent laterally giving the appearance of two lateral lips, each of which bears a sessile lateral, and two prominent head-papillae. The oval capsule is roughly cylindrical and oval on cross-section, while the thickness of the wall varies in different parts. Since this is thicker caudad and thinner caphalad, on its dorsal and ventral than on its lateral aspects, its cavity is, at its cephalad end, wider dorso-ventrad than latero-laterad. The external leafcrown, which originates about one-third of the way down the depth of the mouth capsule, is composed of numerous fine elements originating along a curved line which runs closer to the anterior margin of the buccal capsule dorsally and ventrally than laterally. The dorsal and ventral leaflets are also shorter than the lateral thus giving to the mouth the shape of a dorso-ventral slit. The internal leafcrown forms the anterior margin of the buccal capsule and its elements (leaflets) are short. Tubercles or teethlike structures may be present at the base of the buccal capsule. The oesophagus is short and stout, the cuticle lining the anterior portion may or may not exhibit a plumose sculpturing.

Male: Bursa with well-developed dorsal lobe. Ventral and lateral rays arise from a common trunk, the medio-lateral and postero-lateral separated in their distal portions. The externo-dorsal arises from the base of the dorsal. The dorsal ray is bifurcate for about half its length, and from about the point of bifurcation gives off either two lateral branches arising close together, or a single branch which is cleft to a greater or less extent. Various small projections may be present on the postero-lateral or on the externo-dorsal ray. The spicules are equal, with the points bent in one direction. Gubernaculum present.

Female: Posterior extremity is long and tapering. Vulva is near the anus.

Parasites of elephants, rhinoceroses and warthogs.

TYPESPECIES—*M. murshida*, Lane, 1914. Host—Indian elephant.

Other species from elephants:—

	Host.
M. falcifera (Cobbold, 1882)	Indian elephant
M. indica (Ware, 1924)	,, ,,
M. neveu-lemairei (Witenberg, 1925) ...	,, ,,
M. linstowi, Khalil, 1922	African elephant
M. hadia, Khalil, 1922	,, ,,
M. longicaudata, Neveu-Lemaire, 1928 ...	,, ,,
M. brachyscelis, Mönnig, 1932	,, ,,
M. africana (Lane, 1921)	,, ,,
M. anisa (Khalil, 1922)	,, ,,
M. dawoodi (Khalil, 1922)	,, ,,
M. omoensis (Neveu-Lemaire, 1924)	,, ,,
M. brevicapsulatus (Mönnig, 1932)	,, ,,
M. memphisia (Khalil, 1922)	,, ,,
M. aziza (Khalil, 1922)	,, ,,
M. loxodontae (Neveu-Lemaire, 1928) ...	,, ,,
M. soudanensis (Neveu-Lemaire, 1928 ...	,, ,,
M. brevicaudata (Neveu-Lemaire, 1928) ...	,, ,,
M. lanei? Witenberg, 1925	Indian elephant

DISCUSSION ON CLASSIFICATION AND SYNONYMY.

Witenberg, 1925, created the sub-family MURSHIDIINAE, and takes the genus *Murshidia* out of the sub-family TRICHONEMINAE, Railliet, 1916, on the constant features of the oval shape of the oral aperture with the dorso-ventral axis longer than the lateral, and on the elongation of the leaflets laterally. He proposed to group the two sub-families MURSHIDIINAE and TRICHONEMINAE under a new family TRICHONEMIDAE, without, however, defining the latter. Since there seems to be no special reasons for grouping these two together,

and for taking them out of the STRONGYLIDAE, and since Witenberg himself failed to define this new family TRICHONEMIDAE, I have decided to admit the sub-family, but not to accept the family TRICHONEMIDAE, and to group MURSHIDIINAE on a level with TRICHONEMINAE and the other sub-families of the STRONGYLIDAE as given by Yorke and Maplestone, 1926.

In this sub-family Witenberg includes *Pteridopharynx*, Lane, 1921, *Memphisia*, Khalil, 1922, *Henryella*, Neveu-Lemaire, 1924 and *Buissonia*, Neveu-Lemaire, 1924.

According to Yorke and Maplestone, 1926, the features differentiating the genera *Pteridopharynx* and *Memphisia* from *Murshidia*, Lane, 1914, are not marked enough to constitute a generic distinction. They also conclude that since the chief characters of the mouth capsules in all the species of these genera are the same, *Pteridopharynx*, Lane, 1921, and *Memphisia*, Khalil, 1922, are to be considered as synonymous with *Murshidia*, Lane, 1914.

A detailed study of the species included under *Murshidia*, *Pteridopharynx* and *Memphisia*, leads one to conclude that the inclusion of *Memphisia* and *Pteridopharynx* in *Murshidia* is quite justifiable.

There is no doubt that *Pteridopharynx* and *Memphisia* cannot remain separated. The features used in constituting a generic distinction between them are (1) the presence of a cuticular collar round the anterior end of the body in *Memphisia* and (2) the possession of a branched externo-dorsal ray in the bursa of *Memphisia*.

As regards the cephalic collar in Khalil's figure of *M. aziza*, the cuticular collar can hardly be seen. In *Pteridopharynx brevicapsulatus*, Mönnig, 1932, we find the *Memphisia*-character of the externo-dorsal ray bearing a prominent caudally directed branch, associated with the *Pteridopharynx*-character of "no cuticular collar". Hence considering this species alone, the genera *Pteridopharynx* and *Memphisia* are indistinguishable. The cuticular collar thus appears to be a variable character which cannot be considered as of generic importance.

As regards the branched externo-dorsal ray, this is present in a more or less marked degree in all three genera, and is almost as well pronounced in *Pteridopharynx indica* (Fig. 22) and *Murshidia falcifera* (Fig. 13); as in any species *Memphisia*. So that the value of this as a distinguishing feature between *Murshidia* and *Pteridopharynx* also falls away.

Khalil, 1922, differentiates *Pteridopharynx* from *Murshidia* on the grounds that in the former (1) there is a prominence of the posterior border of the lateral ray and (2) the two external branches are fused almost to their tips.

As regards the prominence of the lateral ray it must be noted that *Murshidia falcifera* as well as *M. murshida*, show a prominence on the posterior border of the postero-lateral ray (Figs. 13 and 8). Although it is never as pronounced as in *P. indica* or *P. brevicapsulatus*, yet it is just as marked as in *P. neveu-lemairei*.

So far as the fusion of the external branches of the dorsal ray is concerned, again it seems to be merely a matter of degree. *M. falcifera* shows them fused for about half their length (Figs. 14 and 16), and this condition is the same in *Pteridopharynx neveu-lemairei* (Fig. 35). In the other species of *Pteridopharynx* and *Memphisia*, this fusion is more complete, leaving only the tips free. So it may be stated that the fusion of the two external branches of the dorsal ray in the three genera shows a gradation from complete separation as in *M. murshida* and *M. brachyscelis*, through a stage of partial fusion as in *M. falcifera*, *M. linstowi* and *P. neveu-lemairei*, to a state of almost complete fusion with only the tips free, as is seen in many of the other species.

Another point raised by Neveu-Lemaire, 1928, against the inclusion of *Pteridopharynx* in the genus *Murshida*, is the width of the body in front of the caudal bursa. In the specimens examined both of *Murshidia* and *Pteridopharynx*, this appears to be a variable character depending on the state of preservation of the material.

The same author also states that the distance between the vulva and anus is considerable in *Murshidia* and practically nil in *Pteridopharynx*. In *P. neveu-lemairei*, however, the anus is well separated from the vulva (Fig. 27). Considering actual distance in millimetres, we find that the vulva and anus in the different species are the following distances apart:—

In *P. omoensis*	=	·075 mm.
,, *P. brevicapsulatus*	=	·09 ,,
,, *P. anisa*	=	·12 ,,
,, *M. aziza*	=	·25 ,,
,, *P. loxodontae*... ...	=	·34 ,,
,, *P. neveu-lemairei*	=	·4 ,,
,, *M. linstowi*	=	·5 ,,
,, *M. hadia*	=	·6 ,,
,, *M. murshida*	=	·72 ,,
,, *M. falcifera*	=	·8 ,,

Here again we find intermediate forms which nullify the difference set up between the two genera.

The possibility whether the presence of a plumose sculpturing on the anterior portion of the oesophagus associated with a general elongate form of the dorsal ray might not be taken as a generic characteristic, shall now be considered.

Were this characteristic to be taken as the chief generic feature of *Pteridopharynx*, *P. africana*, *P. brevicapsulatus* and *M. aziza* would then definitely belong to the genus. The dorsal ray of *P. neveu-lemairei* is much shorter and approaches more or less the condition as found in *M. brachyscelis* the dorsal ray of which is typically that of *Murshidia*, but *M. brachyscelis* also shows the plumose

corrugation of the oesophagus, which is a *Pteridopharynx*-characteristic. *M. brachyscelis* and *P. neveu-lemairei* therefore definitely link up the species of *Pteridopharynx* with the species of *Murshidia* in which a plumose sculpturing of the oesophagus is not present. Thus again it is not possible to separate *Pteridopharynx* from *Murshidia*.

The elongate dorsal ray is also found in *M. aziza*, *M. memphisia* and *P. omoensis*, none of which shows a plumose corrugation of the oesophagus. These species can be linked up with *Murshidia* through *P. neveu-lemaire*, the bursa of which is intermediate between the elongate form of *Pteridopharynx* and the more squat form of *Murshidia*. Hence, it is concluded that *Pteridopharynx* cannot be generically separated from *Murshidia* and that the synonymy of *Pteridopharynx* and *Memphisia* with *Murshidia*, should stand, as proposed by Yorke and Maplestone, 1926.

A Key to the Species of Murshidia.

A. *With anterior portion of oesophagus plumosely sculptured.*

I. *Branch of externo-dorsal ray well pronounced.*

(*a*) Ventral ray cleft for its whole length ... ... **M. indica** ... ... p. 60

(*b*) Ventral ray cleft for half its length ... ... **M. brevicapsulatus** ... p. 68

II. *Branch of externo-dorsal ray rudimentary.*

(*a*) Dorsal ray fairly short and broad, with its external and median branches fused for about half their lengths ... ... **M. neveu-lemairei** ... p. 61

(*b*) Dorsal ray elongate and thin, with its external and median branches completely fused to almost their extreme tips ... ... **M. aziza** ... ... p. 70

III. *With branch on externo-dorsal ray, absent.*

(*a*) Dorsal ray very short, its external branches entirely free, and longer than its median and internal branches ... **M. brachyscelis** ... ... p. 64

(*b*) Dorsal ray very elongate and thin, its external and median branches fused to almost their tips ... ... **M. africana** ... ... p. 65

B. *Plumose sculpturing of the oesophagus, absent.*

I. *Dorsal lobe of bursa short and broad.*

(*a*) *Parasitic in the Indian elephant.*

(i) Oesoph. about 1·0 mm. long, and bases of the external branches of the dorsal ray swollen **M. falcifera** ... ... p. 59

(ii) Oesoph. about 0·6 mm. long, and bases of ext. branches of the dorsal ray not swollen ... ... **M. murshida** ... ... p. 59

(*b*) *Parasitic in the African elephant.*

(i) With a small (±0·1 mm.) curved and more or less S-shaped accessory piece.

(*a*) External leafcrown composed of 40 leaflets ... ... **M. hadia** ... ... p. 63

(*b*) External leafcrown composed of 20-28 leaflets ... ... **M. linstowi** ... ... p. 62

(ii) With a broad (±0·45 mm.) four-sided accessory piece ... ... **M. longicaudata** ... ... p. 64

II. *Dorsal lobe of bursa elongate and thin.*

(*a*) *Teeth present at the base of the capsule.*

(i) Dorsal ray cleft till beyond the origin of the accessory branches **M. dawoodi** ... ... p. 67

(ii) Dorsal ray cleft approx. half way to the origin of accessory branches

(*a*) Cuticular collar present ... ... **M. memphisia** ... ... p. 69

(*b*) Cuticular collar absent ... ... **M. anisa** ... ... p. 66

(*b*) *Teeth absent* ... ... **M. omoensis** ... ... p. 68

NOTE.—Since no males have been found for *M. soudanensis*, *M. brevicaudata* and *M. loxodontae*, these species cannot be included in the key (see descriptions and figures for female characteristics).

Murshidia murshida, Lane, 1914.

The specimens examined, and of which measurements were taken, consisted of a large number of males and females. On the whole the worms examined seemed to be much larger than those examined by Lane, 1914, and Khalil, 1922. (For measurements see Table 1.)

The oral aperture is bounded by 60 leaflets arising about half-way down the depth of the mouth-capsule. According to Lane, 1914, the cervical glands reach to a point 4 mm. caudad of the caudal end of the oesophagus.

Male: The most characteristic features of the bursal rays are the general ruggedness of the outline of the externo-dorsal and dorsal rays (Fig. 5), and the swollen basis of the lateral rays, although the latter characteristic is not always too well pronounced. The three subdivisions of the dorsal rays are situated about equidistant from one another (Fig. 5). The spicules are equal and similar; the shaft is straight for its whole length, the extremity is bent almost directly dorsad, and the extreme end is marked by a prominence or "beak" directed caudad (Fig. 6). An irregular S-shaped accessory piece is present (Lane overlooked the presence of an accessory piece).

Female: The vulva is marked by a fairly long low cuticular prominence caudad of it (Fig. 2). Mature ova were absent from the females examined, but according to Witenberg these measure ·072 × ·048 mm.

Habitat: Large intestine and caecum.

Host: Indian elephant.

Murshidia falcifera (Cobbold, 1882).

Syn. *Strongylus falcifer*, Cobbold, 1882.

Nematode No. 3, Evans and Rennie, 1910.

Strongylus falcifer, Mitter, 1912.

Cylicostomum falciferum, Railliet, Henry and Bauche, 1914.

Murshidia falcifera, Lane, 1914.

The oral aperture is bounded by 80 leaflets. The excretory pore lies a little posterior to the level of the junction of the oesophagus and chyle-intestine and the lateral papillae shortly caudad of this level.

For *M. falcifera* the measurements were also found to be larger on the whole than recorded by Lane, 1914, and Khalil, 1922. (For other measurements, see Table 1.)

Male: The lateral rays of the bursa are not bulbous at the base, and according to Lane, 1914, the dorsal and externo-dorsal rays are not rugged in outline, but an examination of a large number of specimens proved that although the majority have smooth dorsal rays, variations exist, which show a tendency towards ruggedness of the

internal branches of the dorsal rays (Figs. 16 and 17). The externo-dorsal ray has a projection which is a constant feature, but the projection may take in various shapes sometimes having a forked appearance (Fig. 14). Of the three subdivisions of the dorsal ray, the two laterals lie closer together and somewhat separated from the central one (Fig. 16). Some individuals show a tendency towards fusion of the lateral rays of the dorsals, at least on one side.

The shafts of the spicules are curved just cephalad of the terminal bend, giving them a lyrate shape. There is no " beak ". An S-shaped accessory piece is present (Fig. 15) .

Female: Caudal papillae are present between the anus and tip of tail. The cuticle cephalad and caudad of the vulva is but slightly thickened. Ova were not present in the females examined, but according to Lane, 1914, these measure ·05 × ·03 mm. and according to Witenberg, 1925, ·084 × ·041 mm.

Habitat: Large intestine.

Host: Indian elephant.

Murshida indica (Ware, 1924).

Syn. Pteridopharynx indica, Ware 1924.

The material examined consisted of a large number of males and females.

The worms are easily recognised by the featherlike appearance of the anterior portion of the oesophagus (Fig. 21), and the enormous prominence on the ventral side immediately anterior of the vulva in the females (Fig. 26 and 28).

The body is slender, tapering at both ends. The posterior region of the female body is suddenly and markedly constricted to form the tail, which appears to the naked eye as a posteriorly placed spine slightly bent dorsally. Both males and females were perfectly straight; only a few males showed a slight posterior bend; however, none approached the form of a pothook as recorded by Ware, 1924,—this may be due to the different preserving media used.

The head bears two sessile lateral, and four submedian papillae. Two coronal leafcrowns are present. The external leafcrown consisting of 40-42 leaflets, originates near the base of the capsule and its anterior extremity falls just short of the anterior line of the mouth collar. The internal leafcrown (which is not figured by Ware) forms the anterior margin of the mouth capsule—the leaflets are much shorter than those of the external leafcrown.

The mouth collar is separated from the rest of the body. The buccal capsule is typically that of a *Murshidia*, its lateral axis is shorter than its dorso-ventral axis. At the base of the capsule a group of small irregular prominences comparable with teeth, are present (Fig. 24).

Male: The bursa in the male is distinctly divided into a dorsal, median and two lateral lobes. The ventral, lateral and externo-dorsal rays lie close together, while the branches of the dorsal ray are widely

separated from each other (Fig. 22 and 31). The externo-lateral ray is somewhat separated from the other two lateral rays, which lie close together and it is also thicker at the base than the other two (Fig. 23). The postero-lateral ray has an accessory dorsal branch (dorsal boss) which is well marked but stumpy. The externo-dorsal bears an accessory ray, which varies from a pronounced condition in some specimens (Fig. 33) to a mere vestige in others (Fig. 34). The dorsal ray has a very rugged outline in some specimens (Fig. 31), whereas in others it is almost smooth (Fig. 32). The anterior branches of the dorsal ray are slightly bifid at their extremities (Fig. 31), and the posterior branches have pointed extremities and converge slightly; they are subject to variation (Fig. 25). The spicules are slender and equal and provided with fine plumose alae; their posterior extremity is bent dorsally and ends in a fine point (Fig. 36). A saddleshaped accessory piece is present measuring 0·1 mm. × ·05 mm. Ware records well developed prebursal papillae situated slightly in front of the ventral rays, but these could not be identified in the specimens examined. At least no papillae could be found slightly in front of the ventral rays. Three to four anal papillae however, were noticed, situated on the anal cone and surrounding the anal opening laterally and posteriorly.

Female: The openings of the vulva and anus are close together. Immediately anterior to the vulva is an enormous cuticular prominence, which usually covers the vulva (Fig. 26) and posterior to the anus a slight prominence guards the dorsal lip of the anus. The prevulvar prominence was present in most of the females examined, but a number of females was found which showed the post-vulvar (or pre-anal) prominence (Fig. 29) and two other females showed no prominence at all (Fig. 30). All the other features of these latter specimens were similar to those possessing the marked prevulvar prominence, and also their measurements coincided with those of the latter. So that at present these differences may be regarded as variations of the female posterior extremity.

The vaginal measurement varied from 0·56- 0·73 mm. Ova present measured ·056 × ·029 mm. (Ware's measurement for the ova is slightly different.)

(For measurements see Table 1.)

Habitat: Large intestine and stomach.

Host Indian elephant

Murshidia nereu-lemairei (Witenberg, 1925).

Syn. Pterygopharynx nereu-lemairei, Witenberg, 1925.

The material examined consisted of three specimens—one male and two females. (For measurements see Table 1.)

The mouth collar is separated by a slight groove in which are situated the four submedian papillae. According to Witenberg each papilla consists of three parts: a large basal portion; a small middle one and a fine terminal one; this however does not appear to be a constant feature. The head-papillae did not show any distinctive parts, but were the same capitate papillae as are seen in *Murshidia* generally (Fig. 38) except for small prominences situated laterally

near the tips. In the females the head-papillae showed a slight median constriction thus approaching the condition described by Witenberg (Fig. 38), but neither showed the condition as well pronounced as is shown in Witenberg's drawing.

The lateral papillae do not project from the surface of the mouth collar.

The oral aperture is in the form of an elongated oval. The dorsal and ventral leaflets of the external row are shorter than the laterals, giving a crescentic appearance when viewed laterally. The internal leaflets are shorter and relatively inconspicuous, and are represented in Witenberg's figure as a denticulated line. At the base of the capsule very fine tubercles are present (Fig. 37), which are similar to those of *M. falcifera* and *M. murshida*. (These tubercles have not been mentioned by Witenberg.) A dorsal gutter is also present projecting slightly into the mouth capsule. The oesophagus is bottle-shaped, its anterior part is covered with peculiar plates obliquely striated giving it a plumose appearance (Fig. 37), which reach to the level of the nerve ring. These plates measure 0·15 mm. in the male and 0·18 mm. in the female.

Male: In the bursa the dorsal lobe is somewhat longer than the lateral lobes. According to Witenberg the rays are quite smooth, but in the male specimen examined they presented a rather uneven outline (Fig. 35). The spicules are similar and were found to be 1·4 mm. in length. Witenberg's measurement of 0·28 mm. is apparently incorrect for according to his drawing they are much larger. They taper gradually to their terminations and have a feathery appearance. An accessory piece and telamon is present (Fig. 39).

According to Witenberg the anus in the female is protected by a large lip situated caudad, but no prominent lip was seen in the specimens examined (Fig. 27), nor does Witenberg show such an anal lip in his drawing. My measurement for the vagina (0·86 mm.) also differs from that given by Witenberg (0·21 mm.) Mature ova were not present in the females examined but according to Witenberg these are 0·062 mm. long and 0·038 mm. broad.

Habitat: Large intestine.

Host: Indian elephant.

Murshidia linstowi, Khalil, 1922.

Syn. Sclerostomum rectum, Von Linstow, 1907.

Cylicostomum rectum, Gedoelst, 1916.

Murshidia recta, Railliet, Henry and Bauche, 1915.

Description: The body of the male is straight, the female tail is slightly bent ventrally. The mouth collar is well developed and rounded in outline. It is ·07 mm. long and ·2 mm. in diameter. The external leafcrown arises from the inner surface of the mouth collar, two-thirds the way up the capsule, and consists of 28 leaflets (20 according to Gedoelst 1916); these are longer laterally than ventrally to dorsally. Each leaflet tapers to a point. An internal

leafcrown is also figured by Leiper (Fig. 42). Lateral and submedian papillae do not project beyond the head. The mouth capsule is large and globular in shape when seen from the lateral aspect. From a dorsal or ventral aspect it is pearshaped, with a narrow opening. No teeth at the base of the buccal capsule. A cone-shaped oesophageal funnel is present, 0·8 mm. in length.

Female: The wide opening of the vulva is surrounded by a raised cuticular margin. The two uteri end in strong ovejectors. The female tail gradually tapers to a sharp point.

Male: The bursa is very short and indistinctly divided into three lobes. It measures ·27 mm. in length. The ventral ray is bifid throughout its length, and is placed a considerable distance from the ventral edge of the bursa. The lateral rays arise from a common origin. At the site of the bifurcation each ray has a bulblike swelling. The antero-lateral ray lies separate from the other two, and nearer to the ventral rays. The medio-lateral and dorso-lateral rays lie close together. The externo-dorsal has a rugged outline. It arises in common with the dorsal ray. The dorsal ray arises by a very broad stem and bifurcates into two branches, each ending in three distinct rays. The outer two rays arise by a common trunk (Fig. 41). The two spicules are very stout and practically straight except at their tips. The clubshaped tip is bent sharply dorsally. A small curved accessory piece (S-shaped according to Gedoelst) is present, ·13 mm. in length. (For other measurements see Table 1.)

Habitat: Small intestine.

Host: African elephant.

Murshidia hadia, Khalil, 1922.
(Figs. 43-48.)

The body of the male is straight, the female tail is slightly bent ventrally. Mouth collar is well developed and rounded in outline, and is separated from the rest of the body by a distinct groove. The external leafcrown consisting of 40 leaflets arises from the inner surface of the mouth-capsule. These leaflets are longer laterally than ventrally or dorsally. A second leafcrown is not mentioned. The usual 4 submedian head-papillae are present and they project freely above the head, surmounted by a small knob. The wide conical lateral papillae do not project. The cervical papillae are very thin, long and point cephalad. The mouth cavity is large and globular, measuring ·12 mm. in length and ·15 mm. in maximum diameter. The mouth capsule is a massive chitinous structure. The floor of the oral cavity is free of any teeth.

Female: The wide opening of the vulva is surrounded by a raised cuticular margin. The muscular vagina runs straight towards the head end. The tail gradually tapers to a sharp point.

Male: The bursa is very short and indistinctly divided into three lobes of approximately the same length. It measures ·22 mm. in length and ·7 mm. in breadth. The ventral ray is bifid throughout its length. The lateral rays arise from a common origin. At the site of their bifurcation each ray has a bulblike swelling. The

externo-lateral lies separate from the other two, and its ends in a papilla on the external surface, a short distance from the edge of the bursa. The two remaining laterals lie close together. The externo-dorsal has a rugged outline. It arises in common with the dorsal ray. The dorsal ray arises by a very broad origin and bifurcates into two branches, each ending in 3 rays. The outer 2 rays arise by a common trunk and lie close together. The median ray is stouter and longer and ends some distance from the corresponding ray of the opposite side. The dorsal ray is ·25 mm. long.

The two similar spicules are stout and practically straight. The club-shaped point is bent sharply forward. A small curved accessory piece ·1 mm. long is present. (For other measurements see Table 1.)

Habitat: Intestine.

Host: African elephant.

Murshidia longicaudata, Neveu-Lemaire, 1928.

(Figs. 49-52.)

Neveu-Lemaire described this species as follows:

Cylindrical worms, tapering anteriorly in both sexes. The cuticle is only distinctly striated in the anterior portion of the body. The mouth is surrounded by four sub-median head-papillae (lateral papillae are not mentioned). A well developed external corona is present (a second leafcrown is not mentioned). The buccal capsule is cylindrical. Inside the capsule is a distinct prominence. The intestine at its commencement is wider than the oesophagus.

Male: Equally wide anteriorly and posteriorly, giving to the naked eye a truncated appearance posteriorly. Caudal bursa is large. each division of the dorsal ray has three branches. The medio-lateral and postero-lateral rays are irregularly swollen except at their extremity. The spicules are equal, are slightly sinuous and curved and slightly swollen at their extremities. An accessory piece is present.

Female: The tail is not separated off by a notch or constriction. The uterus and vagina contain oval, segmented, thin-shelled eggs. (For measurements see Table 1.)

Habitat: Large intestine.

Host: African elephant.

Murshidia brachyscelis, Monnig, 1932.

(Figs. 53-56.)

Mönnig's description of this species is based on a single male specimen found amongst other nematodes collected from an African elephant. Its main characteristics are as follows:—

A straight worm and much smaller than any of the other species of this genus. The mouth collar is well developed and separated from

the rest of the body by a distinct groove. The external leafcrown consists of 24 leaflets which are shorter dorsally and ventrally than laterally. (A second leafcrown is not mentioned.) The mouth capsule has the shape of an oval ring flattened from side to side and higher laterally than dorsally and ventrally. The "three sharp curved cuticular flanges, one surmounting each of the three muscular oesophageal columns" described by Lane, 1921, in his generic diagnosis of *Pteridopharynx*, are also present here. There is an oesophageal funnel, 0·12 mm. deep, showing the feathered arrangement. The two thin long cervical papillae are directed cephalad.

The bursa is well developed but short. The ventral rays are close together and reach the edge of the bursa. The externo-lateral turns ventrally away from the other laterals and ends in a papilla, which projects slightly beyond the margin of the bursa. The medio- and postero-laterals are parallel. The externo-dorsal is smooth, it arises at the very base of the dorsal stem and runs near to the postero-lateral, not quite reaching the margin of the bursa. The dorsal ray has a broad base which gives off two branches, each again dividing at once into three rays; of these rays the medial one of each side is the shortest. There are two similar alate spicules which are slightly bent, the tips being bent forwards. A small curved accessory piece and telamon is also present.

(For measurements see Table 1.)

Habitat: ?

Host: African elephant.

Murshidia africana (Lane, 1921).

(Figs. 57-60.)

Syn. Pteridopharynx africana, Lane, 1921.

Description: Rather slender worms, widest at about the middle, with a discoidal head. The leaflets of the corona are 16 in number. The mouth capsule is nearly circular, wider than long. There are two prominences projecting into the floor of the mouth capsule, which Khalil, 1922, regards as teeth, and Lane, 1921, as the beginning of an oesophageal funnel. The oesophagus is short and wide, constricted at the site of the nerve ring. The anterior portion of the oesophagus has a plumose appearance, ·15 mm. long.

Male: The bursal rays are long and slender. The outer branches of the dorsal ray are united up to near their tips (Fig. 60). The dorsal ray of the bursa may have an irregular outline. The spicules are similar and equal, each with a central rod, which ends in a curve and terminates in a sharp hook and with two thin alae, plumosely marked.

Female: In the female caudal papillae are present at a distance of ·55 mm. from the tip of the tail.

(For other measurements see Table 1.)

Habitat: Stomach.

Host: African elephant.

Murshidia anisa (Khalil, 1922).

(Figs. 61-64.)

Syn. Pteridopharynx anisa, Khalil, 1922.

Body of both male and female is practically straight with the greatest diameter about the middle. The body tapers slightly towards the head-end and caudal end. The female tail is suddenly constricted from the body, appearing to the naked eye as a spinous process projecting from the posterior end of the body. The skin is finely striated throughout the whole length of the body. The mouth collar is separated from the rest of the body by a distinct groove. It measures ·125 mm. in diameter and ·02 mm. in length. The external leafcrown consists of 20 leaflets which, when viewed from the ventral aspect forms a crest, being longer in the centre than at either side. Cephalic glands are well marked. Four submedian and two lateral papillae are present. The cervical papillae are long and thin. The mouth capsule is longer than broad. A chitinous plate forms the wall of the posterior half of the mouth capsule, and a funnel-like extension of it forms the wall of the anterior portion. From the posterior end of this chitinous ring projects a series of sharp teethlike processes, overhanging the opening of the oesophagus. The oesophagus is hardly constricted at the site of the nerve collar.

Male: The bursa is long and indistinctly separated into three lobes. The elongation is due to the dorsal lobe. The bifid ventral ray is short and placed close to the cephalic end of the bursa. The lateral rays arise from a common trunk, and in addition to the three ordinary branches has a fourth stumpy-looking ray which is directed caudally. The externo-dorsal is stout and arises close to the lateral rays; it does not reach the edge of the bursa. The dorsal ray is ·7 mm. long. It gives off a branch on either side after ·18 mm. The ending of each of these branches is bifid. (This bifurcation is longer than in *M. africana*). The main stem of the dorsal ray divides into two branches, which end at the posterior limit of the bursa. The bursa is ·82 mm. long and ·47 mm. broad. The spicules are similar, their cephalic ends are thick and surrounded by an everted lip. In their course they entwine each other. Their tips are sharp and curve backwards. An accessory piece, 0·13 mm. long, is present, and is curved dorso-ventrally.

Female: At the level of the vulva the ventral surface curves towards the dorsum forming a conical-shaped tail. On the tail are two caudal papillae, marked on the surface by a depression ·33 mm. from the extremity of the tail.

(For measurements see Table 1.)

Habitat: Intestine.

Host: African elephant.

Murshidia dawoodi (Khalil, 1922).

(Figs. 65-68.)

Syn. Pteridopharynx dawoodi, Khalil, 1922.

The bodies of both males and females are practically straight, tapering slightly towards both ends. In the female the tail becomes suddenly constricted from the body, appearing to the naked eye as a spinous process projecting from the posterior end of the body. The mouth collar has a rounded outline and is separated from the rest of the body by a distinct groove. It is ·015 mm. in diameter and ·30 mm. in length. The coronal leaflets have a conical outline when seen from the lateral aspect. The point of the cone is rounded and projects above the outline of the mouth.

Four capitate submedian, and two lateral papillae are present. The cervical papillae are long and thin. The mouth capsule is pear-shaped. It is longer than broad. A chitinous plate forms the wall of the deep part of the mouth capsule and a thin expansion from it forms the funnel-shaped wall of the outer part. The chitinous plate is ·1 mm. in length. From the posterior end of the chitinous ring projects a series of sharp tooth-like processes overhanging the opening of the oesophagus. The oesophagus is slightly constricted at the site of the nerve collar. There is a conical shallow oesophageal funnel bordered by a thick cuticular edge. The excretory vesicle is a fairly fusiform sac with a short duct.

Male: The bursa is distinctly separated into three lobes. The dorsal lobe is longest. The two branches of the ventral ray lie close together. The lateral rays arise by a common stem. The externo- and medio-lateral lie wide apart. The postero-median has a rounded thickening in its posterior border close to its base. The externo-dorsal ray is stout, and arises close to the lateral rays. It does not reach the edge of the bursa. The dorsal ray is comparatively long (0·45 mm.). It divides into two principal branches, each of which in its turn gives off a thick lateral branch. These lateral branches divide in their distal two-fifths, but their subdivisions lie close to one another. There is a distinct truncated genital cone. The two similar and equal spicules are very long; their cephalic ends are thick and surrounded by an everted tip. The tips are bent backwards but end in a fine point, quite different from the club-shaped tips of the spicules in other species of the genus *Murshidia*. An accessory piece is present—·25 mm. long.

Female: The vulval opening is directed caudally lying on a large protruding papilla. The two muscular ovejectors open into the vagina, connecting it with the corresponding uterus.

(For other measurements see Table 1.)

Habitat: Small intestine.

Host: African elephant.

Murshidia omoensis (Neveu-Lemaire, 1924).

Syn. Pteridopharynx omoensis Neveu-Lemaire, 1924.

The description which follows is as given by Neveu-Lemaire for *M. omoensis* from the African rhinoceros, which he stated is indistinguishable from *M. omoensis* from the African elephant.

Whitish cylindrical worms with fine cuticular striations. The terminal mouth is surrounded by the usual four sub-median, and two lateral head-papillae, each surmounted by a small prolongation in the form of a button. The external leafcrown is formed of converging leaflets of variable length, commencing at the posterior margin of the capsule, and converging at their free tips. There is also an internal leafcrown, the leaflets (teeth) of which are short and annular and form the margin of the anterior border of the buccal capsule. (Neveu-Lemaire does not figure it in his specimens from the African elephant, but see Fig. 72.)

Cervical glands are well developed. The oesophagus is very short and globular and constricted at the level of the nerve ring. Valves are present at the origin of the intestine.

The anterior extremity of the female is truncate, the posterior extremity is very tapering. The vulva and anus are very close together. (Fig. 71.)

Neveu-Lemaire states that he found male specimens of the above species in both African rhinoceros and elephant, but he does not describe the male. His figure (Fig. 70) of the male bursa, however, shows a dorsal boss attached to the medio-lateral ray. If his figure is correct, this appearance is apparently of rare occurrence, since in *Pteridopharynx* this dorsal boss is usually on the postero-lateral ray, and not on the medio-lateral.

(For measurements see Table I.)

Habitat: Large intestine.

Host: African elephant and African rhinoceros.

Murshidia brevicapsulatus (Mönnig, 1932).

(Figs. 73-77.)

Syn. Pteridopharynx brevicapsulatus, Mönnig, 1932.

The main characteristics as described by Mönnig are as follows:

Practically straight worms not tapering much towards the extremities except in the case of the female tail. The mouth collar is separated from the rest of the body by a groove, and bears four prominent submedien and two broad, short, lateral papillae. The cuticle is striated except on the head where it is smooth. The external leafcrown arises from the lining of the buccal capsule and consists of 40 leaflets which are ·047 mm. long laterally and shorter dorsally and ventrally. (A second leafcrown is not mentioned.) Cuticular flanges are present at the base of the capsule. The oesophageal funnel shows a feathered arrangement and is ·14 mm. deep.

Male: The bursa is well developed showing a relatively long dorsal lobe. The ventral rays have a common stem which is about as long as each branch. The latter are close together and do not quite reach the edge of the bursa. The three lateral rays diverge slightly from one another and the postero-lateral bears a well developed backward projection at its base. The externo-dorsal ray arises at the base of the dorsal stem and runs outwards with a slight backward curve. At its base there is a small backwardly directed knob and slightly more distally it bears a prominent caudally directed branch. The dorsal ray gives off a branch on each side before it bifurcates, these two branches have bifid tips, showing that they are made up of two fused branches each. The terminal dorsal branches bear a number of irregular prominences on the lateral aspect of their bases and end near to the margin of the dorsal lobe. There are two equal alate spicules, more or less triangular with the tips bent sharply ventral-wards. A curved accessory piece and small telamon is present.

Female: The tail is fairly long, bent ventrad and acutely pointed. (For measurements see Table I.)

Habitat: ?.

Host: African elephant.

Murshidia memphisia (Khalil, 1922).

(Figs. 78-82.)

Syn. Memphisia memphisia, Khalil, 1922.

Description: Slender straight worms. The body is slightly attenuated at the anterior end. Just posterior to the mouth is an expansion of cuticle, which encircles the body, and is referred to as the cuticular-collar. The tail of the female is suddenly constricted from the body, appearing to the naked eye as a large posterior placed spine. The cuticle is striated, and towards the anterior end of the body becomes inflated, forming in optical section, two alae, which surround the whole circumference of the body.

The mouth collar is indistinctly separated from the rest of the body. It is ·02 mm. long and ·12 mm. in diameter.

The anterior leafcrown consists of 36 incompletely separated leaflets. The usual six head-papillae are present. Cervical papillae are long and delicate. Seen from the ventral surface, the mouth capsule is practically quadrangular, being slightly broader at the outlet. The chitinous ring surrounding the oral cavity is thick and nearly vertical. There are two teeth projecting into the base of the mouth cavity.

The oesophagus is short and broad and a shallow oesophageal funnel is present. It is slightly constricted at the site of the nerve collar.

Male: The bursa is elongated and distinctly divided into three lobes. The bursa is ·75 mm. long and ·55 mm. broad. The dorsal lobe is about double the length of the lateral lobes. The ventral,

lateral and externo-dorsal rays are crowded together, while the branches of the dorsal ray are wide apart. The bifid ventral ray is placed near the anterior edge of the bursa. The lateral in addition to the usual three branches gives off a 4th stumpy ray, directed caudally. The externo-dorsal ray does not reach the edge of the bursa. Near its origin it gives off a long branch directed caudally and having a rounded extremity. It is ·1 mm. long. The dorsal ray is ·7 mm. long. It gives off a branch on either side before ultimately dividing into two rays. The first two branches have a double papilla at their end. The terminal branches are a long distance apart at the caudal extremity of the bursa.

The spicules are similar and thin with a fine termination, bent dorsally. There is a short accessory piece, ·1 mm. long, curved on itself.

Female: The vulval opening is surrounded by a prominent lip especially on the caudal side, and this latter separates it from the anus.

(For other measurements see Table I.)

Habitat: Intestine.

Host: African elephant.

Murshidia aziza (Khalil, 1922).

(Figs. 83-87.)

Syn. Memphisia aziza, Khalil, 1922.

Description: Slender worms with a narrow head end (·1 mm. in diameter). There is no cuticular expansion. The cuticle is finely striated. The mouth collar is separated from the rest of the body by a shallow groove. It is irregularly rounded when seen laterally, and almost quadrangular when seen ventrally or dorsally. The latter appearance is due to the presence of the lateral head papillae being on the extreme edge of the mouth collar. It is ·02 mm. long and ·1 mm. in diameter. The usual six head-papillae are present. The external leafcrown is composed of 24 leaflets which surround the oval-shape mouth opening. The cervical papillae are thin. The mouth capsule is longer than broad. The chitinous ring surrounding the oral cavity is very thick and almost rectangular in optical section. At the base of the capsule there is a ring of 24 sharp teeth. There are four cephalic glands surrounding the anterior part of the oesophagus from the level of the nerve collar to the mouth capsule.

The oesophagus has a plumose appearance. A shallow oesophageal funnel, surrounded by a thick chitinous wall, is present. The oesophagus is slightly constricted at the site of the nerve ring.

Male: The bursa is distinctly divided into three lobes. The dorsal lobe is much longer than the lateral. The bursa resembles that of *M. memphisia.* It is ·5 mm. in length and ·45 mm. in breadth. The ventral and lateral rays are crowded together. The 4th division (but Khalil does not figure this) of the lateral ray is shorter and knoblike. The externo-dorsal ray does not reach the edge of the bursa. It has a small projection near its origin. The dorsal ray is ·47 mm. long. Each of its first two branches terminate in two

papillae. The terminal two branches terminate close to each other at the caudal end of the bursa. Fairly long pre-bursal papillae are present, ·7 mm. from the caudal end of the bursa. The spicules are similar. Their tips are curved, pointed dorsally. The spicular-sheath is transversely striated in its upper third. The accessory piece is ·1 mm. long enclosing the spicules.

Female: The tail gradually tapers to a point. There are two caudal papillae marked by a slight depression in the lateral line. These are placed ·14 mm. from the tip of the tail. The ova are thin shelled.

(For other measurements see Table I.)

Habitat: Intestine.

Host: African elephant.

Murshidia loxodontae (Neveu-Lemaire, 1928).

(Figs. 88-90.)

Syn. Memphisia loxodontae Neveu-Lemaire, 1928.

Only the females of this species were found and described by Neveu-Lemaire. The body is cylindrical tapering slightly in front, cuticular striations are only slightly marked. A narrow terminal mouth is present. The external corona formed of convergent teeth (leaflets), the internal corona fairly well developed. Four submedian and two lateral papillae are present. The buccal capsule is cylindrical. Cephalic glands are well developed. The oesophagus is but slightly swollen in its posterior third. The body shows a sudden narrowing at the level of the anus. The vagina is fairly long.

(For measurements see Table I.)

Habitat: Large intestine.

Host: African elephant.

Murshidia soudanensis (Neveu-Lemaire, 1928).

(Figs. 91-93.)

Syn. Memphisia soudanensis Neveu-Lemaire, 1928.

Only the female of this species was found by Neveu-Lemaire and described as follows:

Body cylindrical tapering anteriorly, cuticular striations are feebly marked anteriorly, but become more marked posterior to the oesophagus. The mouth is terminal, surrounded by four submedian papillae. The external corona consists of numerous delicate leaflets. The buccal capsule is somewhat coneshaped with the largest diameter anteriorly. Cephalic glands are well developed. The oesophagus is hardly swollen in its posterior third. The posterior extremity is suddenly truncated at the level of the anus and vulva, which are situated at the same level. Behind the anus is a short narrow tail, slightly curved ventrally.

(For measurements see Table 1.)

Habitat: Large intestine.

Host: African elephant.

Murshidia brevicaudata (Neveu-Lemaire, 1928).

(Figs. 94-96.)

Syn. Memphisia brevicaudata, Neveu-Lemaire, 1928.

The description as given, is taken from Neveu-Lemaire:—

The body is cylindrical, tapering anteriorly. The cuticular striations are feebly developed anteriorly, but are more marked posteriorly. The mouth is terminal, surrounded by four submedian papillae. The external corona is narrow; the teeth (leaflets) converging at their free ends. [In Neveu-Lemaire's figure of *M. brevicaudata*, (Fig. 95),the anterior edge of the mouth capsule is serrated—this may be taken as a possible indication of the presence of an internal leafcrown. It is however, not shown for *M. soudanensis*.]

The buccal capsule is roughly coneshaped and widest anteriorly. Cephalic glands are well developed. The oesophagus is swollen in its posterior third. The posterior extremity is broad and suddenly truncated at the level of the anus and vulva, both are situated at the same level, 0·1 mm. apart. Behind the anus is a narrow tail separated from the body by a notch. The ovejectors are well developed, and the vagina is short.

Habitat: ?

Host: African elephant.

M. brevicaudata and M. soudanensis; Neveu-Lemaire distinguishes *M. brevicaudata* from his *M. soudanensis* and from the other species of the same genus, by the shape of the tail. The other characters of the two worms are exactly similar according to his descriptions and as shown by his drawings (compare Figs. 91-96). The measurements also coincide almost exactly except for small differences. (See Table 1.) As regards relative proportions the two worms are almost identical, as the following figures show:—

	M. soudanensis	*M. brevicaudata.*
Tail to body length	0·029 : 1	0·026 : 1
Max. diameter to body length	0·036 : 1	0·035 : 1
Oesoph. to body length	0·028 : 1	0·029 : 1
Diameter of Oesoph. to length of Oesoph.	0·363 : 1	0·450 : 1
Length of nerve-ring from anterior extr. of oesoph., to length of oesoph.	0·345 : 1	0·300 : 1
Length of vulva from tail-end to body-length	0·036 : 1	0·032 : 1
Width of ova to length of ova	0·583 : 1	0·581 : 1

Only three specimens of *M. brevicaudata* and four specimens of *M. soudanensis*, and no males were found, and all in the same host. It is doubtful whether the slightest difference between the extremities of the two worms is a constant specific character. It is more likely that both are variations of the same species. In other species of *Murshidia* the posterior extremity of the female was also found to vary among individuals of the same species, collected in the same individual

host. This was particularly the case in the female of *M. indica*. Here a cuticular prevulvar prominence is a characteristic feature; however, individuals were found in which this prominence was absent, and in which a more or less marked post-vulvar prominence then tended to appear, the rest of the female characters being identical. (See Figs. 26, 28, 29, 30.)

We can thus assume that *M. brevicaudata* and *M. soudanensis* are variations of the same species.

Murshidia lanei, Witenberg, 1925.

This species was described on material consisting of *one* specimen (male) only, found by Witenberg 1925 among some *Murshidia*. The main characteristics on which this species is founded are as follows:—

The worm measures 21 mm. in length with maximum diameter of ·6 mm. The oesophagus measures ·50 mm. × ·35 mm. The distance of the excretory pore from the anterior extremity measures 1·0 mm. Each half row of leaflets consists of 24 elements (i.e. 48 in all). The leaflets are acutely pointed, they increase in length gradually from the end of each row to its middle, but the four central leaflets are exceedingly long and project considerably over the line (Fig. 97). The shortest leaflets reach almost to the level of the anterior margin of the body, whilst the highest ones reach the level of the tips of the sub-median papillae.

The spicules are similar without clearly visible alae and taper gradually and bend dorsally; they contain a canal-opening on their tips. There is a small spine behind the opening of this canal on the tip of each spicule. (In its essentials the spicules do not differ radically from those of *M. murshida*.) The accessory piece presents the shape of an irregular S., similar to *M. murshida*. A single small papilla projects in front of the cloacal opening. A telamon is present.

Discussion: The measurements and features of the male bursa, spicules, and of the mouth capsule are seen to coincide with those recorded as characteristics for *M. murshida*. Although Witenberg figures a very short internal leafcrown along the anterior edge of the capsule, he makes no mention of it in his description. The presence of this internal leafcrown makes these mouthparts fall into line with those of *M. murshida*.

As regards the four central leaflets, in a large number of *M. murshida* males and females examined, marked irregularities were found in the relative lengths of the external coronal leaflets, in some cases approaching the condition as described by Witenberg for *M. lanei*. These irregularities in the coronal leaflets were more pronounced in badly preserved specimens and in partly squashed ones.

On the preceeding facts it is therefore doubtful whether the one specimen described by Witenberg presents specific differences from *M. murshida*. Until more convincing information is obtained, it would perhaps be more correct to consider this coronal structural peculiarity as an irregularity in the corona of *M. murshida*. So that at the most, one could consider this condition as a variety of the species *M. murshida*. On these grounds it is proposed to discard the species *M. lanei*.

TABLE I.

Host.	*M. murshida.* Indian Elephant.		*M. falcifera.* Indian Elephant.		*M. indica.* Indian Elephant.		*M. neveu-lemaire* Indian Elephant.	
	♂	♀	♂	♀	♂	♀	♂	♀
Total length	20–23	24–28·[illegible]	22–28·8	29–36·6	16·7	21·2	25	25·27·3
Maximum diameter	·75	·9	1·02	1·17	·60	·86		
Diameter of head	·18	·21–·25	·27	·28–·32	·18		·20	·25
Diameter of Capsule x depth	·07–·10		·11–·15		·055	·06	·06	·07
No. of leaflets (Ext.)	60		80		40–42		44 app.	
Length of oesophagus	·5–·6	·5–·7	·9–1·1	·9–1·2	·57	·62	·68	·7
Maximum diameter of oesophagus	·22	·27	·36	·36–·45	·2	·23	·36	·36
Nerve ring from ant. end	·2–·4		·5		·3		·35	·36
Excr. pore from ant. end	1·0		1·41		1·04	1·1	1·09	1·18
Cervical papillae from ant. end	1·10		1·55		1·04–1·31		1·09	1·27
Cuticular striations	·007		·008		·0016		·006	
Length of Spicules	1·1–1·3		1·5–2·1		1·06		1·4	
Length of vagina		1·8		1·81–2·22		·56–·73		1·86
Vulva from anus		·72–·86		·81–1·27		·14		·35–·4
Anus from tail end		1·63		1·71–2·27		·72		1·2
Ova		·072x·048		·084x·041		·056x·029		·062x·038

Host.	*M. linstowi.* African Elephant.		*M. hadia* African Elephant.		*M. longicaudata* African Elephant.		*M. brachyscelis* African Elephant.
	♂	♀	♂	♀	♂	♀	♂
Total length	21–26·5	25·8–29·5	18·5	24	23	28	8·3
Maximum diameter	·8	·85	·67	·82	1·0	1·2	·3
Diameter of head			·2		·19	·23	·16
Diameter of capsule x depth	·13x·07–·08		·15x·07		·13x·05	·15x·17	
No. of leaflets (Ext.)			40				24
Length of oesophagus	·9–1·0		·85	·9	·59	·6–·7	·46
Maximum diameter of oesophagus	·33		·26	·34	·24	·275	
Nerve ring from ant. end	·42–·45		·6		·3 app.		·3 app.
Excr. pore from ant. end	1·23–1·6		·68				·73
Cervical papillae from ant. end	·95–1·41		1·14	1·4			·85
Cuticular striations	·0094		·009				·009
Length of spicules	·96–1·02		1·1		1·65		·75
Length of vagina						·8	
Vulva from anus		·5–·9		·6		1·0	
Anus from tail end		2·3–3·15		2·25		2·55–3·10	
Ova		·05x·038		·04x·023		·07x·05	

(All measurements given in millimetres.)

TABLE I—(*continued*).

HOST	*M. africana* African Elephant. ♂	♀	*M. anisa* African Elephant. ♂	♀	*M. dawoodi* African Elephant. ♂	♀	*M. omoensis* African Elephant. ♂	♀	*M. brevicapsulatus* African Elephant. ♂	♀
Total length	17	18	14–15	16·5–17	23	28	15	18	13	16·8
Maximum diameter	·55	·62	·46	·65	·7	9	·65	·85	·43	·59
Diameter of head							·2–·25			
Diameter of capsule x depth			·065x·093		·1x·135		·1x·04			·17x·02
No. of coronal leaflets (Ext.)			20		24					
Length of oesophagus	·57		·45	·57	·87	·95	·4–·5		·52	·54
Maximum diameter of oesophagus			·14		·24	·3	·21			·23
Nerve collar from ant. end	·3		·25		·42	·45	·23		·25	·27
Excr. pore from ant. end	·97		·87		1·22	1·3			·79	·83
Cervical papillae from ant. end	1·1		·92		1·1				·98	
Cuticular striations	·014		·01		·01–·03				·011	
Length of spicules	·9		·82		1·65		·75		·98	
Length of vagina		·55								
Vulva from anus		·2		·12		·8		·075		·09
Anus from tail end		1·03		·52		1·4		1·025		1·0
Ova		·062x·032	·048x·03			·065x·035	·06x·03			

HOST.	*M. memphisia* African Elephant. ♂	♀	*M. aziza* African Elephant. ♂	♀	*M. loxodontae* African Elephant. ♂	♀	*M. soudanensis* African Elephant. ♀	*M. brevicaudata* African Elephant. ♀
Total length	13·5–14	14·8–16	12	15	17		19	17
Maximum diameter	·55		·5	·5	·77		·7	·6
Diameter of head			0·1		·15		·21	·20
Diameter of capsule x depth	·09x·05		·072x·045		·075x·06		·11x·07	·10x·07
No. of coronal leaflets (Ext.)	36		24					
Length of oesophagus	·48		·47		·45		·55	·50
Maximum diameter of oesophagus	·18		·14		·16		·2	·20
Nerve ring from ant. end	·25		·2		·26		·28	·24
Excr. pore from ant. end			·72					
Cervical papillae from ant. end	·92		·82					
Cuticular striations	·01		·01					
Length of spicules	·9		·75					
Length of vagina		1·7		·52				
Vulva from anus		·08		·25	·34		·12	·10
Anus from tail end		·58		·66	·64		·57	·45
Ova		·055x·035		·06x·035	·06x·035		·06x·035	·055x·032

(All measurements given in millimetres.)

SUBFAMILY II, TRICHONEMINAE Railliet, 1916.

Syn. Cylistominae Railliet, 1915.

Subfamily Diagnosis: Strongylidae, without a transverse ventral cervical groove, or cephalic vesicle; buccal capsule cylindrical and as a rule short or ringshaped. Dorsal gutter relatively short or absent, never reaching the anterior margin of the buccal capsule.

Parasites of the alimentary tract of Vertebrates.

Genus QUILONIA Lane, 1914.

Syn. Evansia, Railliet, Henry and Joyeux, 1913.

Nematevansia, Ihle, 1919.

Quilonia, Ihle, 1919.

Paraquilonia, Neveu-Lemaire, 1924.

Generic Diagnosis: Fairly slender worms. Mouth terminal, surrounded by a mouth collar bearing four prominent sub-median, and two sessile, lateral head-papillae. The leaflets of the anterior (external) leafcrown are few and characteristically curved, and surround the mouth anteriorly. A second leafcrown is present or absent. The mouth cavity is narrower than the buccal capsule—the latter is extremely short. One, two or more teeth may be present at the base of the buccal capsule. The oesophagus is nearly cylindrical in shape.

Male: The bursa is divided into three lobes. The dorsal lobe tends to be longer than the lateral lobes. The dorsal ray is bifurcate, each branch having three subdivisions. The spicules are equal and similar, and each has a sickle-shaped point. The accessory piece is curved from side to side, the concavity being ventrad.

Female: The posterior extremity is straight, long and pointed. The vulva is situated in the caudal third of the body. The ovejectors are divergent, but the posterior uterus immediately turns cephalad. Then the two uteri run cephalad side by side.

Parasites of the elephant and rhinoceros.

Type-species: Quilonia renniei (Raill, Henry and Joyeux 1913) (from Indian Elephant).

Other species from elephants:—

Q. travancra, Lane, 1914	In Indian	elephant.	
Q. apiensis (Gedoelst, 1916)	,, African	elephant.	
Q. africana, Lane, 1921	,,	,,	,,
Q. uganda, Khalil, 1922	,,	,,	,,
Q. brevicauda, Khalil, 1922	,,	,,	,,
Q. ethiopica, Khalil, 1922	,,	,,	,,
Q. khalili, Neveu-Lemaire, 1928 ...	,,	,,	,,
Q. loxodontae, Neveu-Lemaire, 1928	,,	,,	,,
Q. magna, Neveu-Lemaire, 1928 ...	,,	,,	,,

A Key to the Species of Quilonia.*

A. *Coronal leaflets project above the head.*

I. *Buccal teeth present.*

(*a*) Buccal capsule bears a single bulbous tooth ... ... **Q. khalili** ... p. 81

(*b*) Buccal capsule bears two or more teeth.

(1) Internal sub-branch of the dorsal ray longer than the median and external sub-branches ... ... **Q. apiensis** ... p. 79

(2) External sub-branch of the dorsal ray longer than the median and internal sub-branches ... ... **Q. uganda** ... p. 80

(3) Sub-branches of the dorsal ray very short and equal ... **Q. magna** ... p. 82

II. *Buccal teeth absent.*

(*a*) Coronal leaflets 18 in number ... **Q. renniei** ... p. 77

(*b*) Coronal leaflets 26 in number ... **Q. loxodontae**... p. 82

(*c*) Coronal leaflets ±10 in number **Q. travancra** ... p. 78

B. *Coronal leaflets on a level with, or do not project above the head.*

I. *Buccal teeth present.*

(*a*) Buccal teeth two in number ... ... **Q. africana** ... p. 79

(*b*) Buccal teeth three in number ... **Q. brevicauda**... p. 80

II. *Buccal teeth absent.*

(*a*) A second corona present, with cleft leaflets ... ... **Q. ethiopica** ... p. 81

Quilonia renniei (Raill; Henry and Joyeux, 1913).

(Figs. 98-101.)

Syn. *Nematode No. 2 from Indian elephant*, Evans and Rennie 1910.

Evansia renniei Railliet, Henry and Joyeux, 1913.

Quilonia quilona Lane, 1914.

Evansia renniei Lane, 1915.

Nematevansia renniei Ihle, 1919.

Quilonia renniei Ihle, 1919.

Description: The main features of this species are: The leaf-crown projects freely above the head. The coronal leaflets are thin and long. The dorsal ray of the bursa is comparatively short (0·35

* This key is constructed on the descriptions and figures given by the original authors, since, with the exception of a single specimen of the species *Q. travancra*, the material studied by the present writer contained no species belonging to the genus *Quilonia*.

mm. in total length). The dorsal ray bifurcates in its posterior third. There is a rather long common stem before the trifurcation. The lateral sub-branch comes off first, and does not reach the edge of the bursa. The median and internal sub-branches are fused until practically at their tips.

In the female caudal papillae are present, 1·2 mm. from the tip of the sharp pointed tail.

(For other measurements see Table 2.)

Habitat: Caecum.

Host: Indian elephant.

Quilonia travancra Lane, 1914.

(Figs. 102-106.)

Syn. Evansia travancra Railliet, Henry and Bauche, 1915.
Nematevansia travancra Ihle, 1919.

A single male specimen was encountered amongst the material; but although the structure of its caudal bursa no doubt places it into the species *Q. travancra*, it nevertheless does not conform in all respects to the description given for *Q. travancra* by Lane 1914. The latter author describes the worm as follows:—

The mouth is surrounded by 10 rays which do not project beyond the head. The bursal rays are all stouter and the dorsal ray also longer, than those of *Q. renniei*. The dorsal ray is 0·85 mm. long. The dorsal lobe is not marked off from the lateral lobe. The dorsal ray bifurcates in its posterior third. The three sub-branches come off practically at the same level, and are approximately of the same length. The spicules are stouter than those of *Q. renniei*, and wavy in outline. In the female the lateral caudal papillae are 0·9 mm. from the tip of the tail.

(For other measurements recorded by Lane, see Table 2.)

The male specimen studied by the present writer differs from Lane's description of *Q. travancra* on the following points:

The coronal leaflets project freely beyond the head (See Fig. 102).

The spicules are well developed, and are *not wavy* in outline as stated by Lane, 1914.

The cervical papillae are situated anterior to the excretory pore, and Lane, 1914 states that these organs are equidistant from the anterior end.

It also differs slightly in certain measurements, as the following measurements will show (Compare with Lane's measurements in Table 2).

Total length	19·1	mm.
Maximum diameter	0·624	,,
Diameter of head	0·244	,,
Number of coronal rays	±10	

Length of buccal capsule	0·048 mm.
Diameter of buccal capsule	0·128 ,,
Length of oesophagus	0·83 ,,
Maximum diameter of oesophagus	0·25 ,,
Cervical papillae to anterior end	0·81 ,,
Excretory pore to anterior end	0·82 ,,
Nerve ring to anterior end	0·40 ,,
Length of spicules	0·912 ,,
Length of accessory piece	0·16 ,,

Habitat: Intestine.

Host: Indian elephant.

Quilonia apiensis (Gedoelst, 1916), Lane, 1921.

Syn. Evansia apiensis Gedoelst, 1916,

Description: This species was fully described by Khalil 1922, from material consisting of 25 specimens. Khalil describes the body as tapering towards either end. The mouth collar, irregularly rounded in outline, is distinctly separated from the rest of the body by a groove. There is a curious cuticular prolongation in optical section, in the form of a spine at the site of the groove (Fig. 107). The external leaflets consist of 12 in number, and project above the head (Fig. 107). The internal leafcrown consists of 12 leaflets, which have rounded blunt ends and do not project above the mouth collar. The chitinous buccal capsule is short and ringshaped. The oral cavity is coneshaped, wide laterally and contracted from before backwards. A small oesophageal funnel is present. From the top of the oesophageal columns two spinous processes project into the mouth capsule (Fig. 109).

Male: The posterior lobe (dorsal lobe) is slightly longer than the lateral lobes. The bifid ventral ray lies close to the lateral rays (Fig. 111). The externo-dorsal ray arises from the stem of the dorsal ray (Fig. 112). The dorsal ray is ·7 mm. long, and appears rather broad and short. It bifurcates in its lower third. Each branch trifurcates at the same level, the sub-branches being relatively long with the internal sub-branch the longest.

Female: The tail of the female is rather broad, with a fine tip. As in most species of *Quilonia*, the vulval opening is covered by a brown cement.

(For measurements see Table 2.)

Habitat: Intestine.

Host: African elephant.

Quilonia africana Lane, 1921.

Description: In this species the corona is sunken and consists of 10 massive rays. The two sub-ventral teeth are long and sharp (Fig. 114).

The bursal rays are fairly delicate. The cleft between the medio-lateral and dorso-lateral is deeper than that between the medio-lateral and externo-lateral (Fig. 116). The dorsal ray is long and slender and bifurcates in its lower third. Each branch trifurcates after some distance, the lateral sub-branch coming off first, and the median subbranch much later; the three branches diverging from one another (Figs. 115 and 118). In one specimen examined by Khalil, 1922 the externo-dorsal ray was completely missing on one side (Fig. 115). The spicules have fine shafts and delicate striated alae.

The tail of the female is exceptionally long and the point is very fine.

(For measurements see Table 2.)

Habitat: Stomach.

Host: African elephant.

Quilonia uganda Khalil, 1922.
(Figs. 119-123.)

Description: The females are longer and thicker than the males. The mouth collar is rounded in outline and distinctly separated from the body by a groove. The external leafcrown consists of 12 leaflets, fairly broad and characteristically bent at their tips. They project a little distance above the head. The internal leafcrown is composed of 12 short stumpy leaflets, which have a rounded end, and which do not project above the head. From the floor of the capsule project two long chitinous teeth, each surmounted by a delicate sharp spine.

The dorsal lobe of the male bursa is slightly longer than the lateral lobes and has a rounded end (Fig. 123). The dorsal ray is long and thin, being ·5 mm. in length. It bifurcates higher than its posterior third, thus giving each branch (which is long and slender) a longer common stem before the trifurcation. The lateral branch comes off first, all three sub-branches are short and slender, the lateral one being longest. The other rays are all fairly slender. The pre-bursal papillae are placed ·52 mm. from the tip of the bursa. The tips of the spicules are sharp and bent ventrally.

The female tail ends in a fine point. The anal opening is marked off from the surface by a round depression.

(For measurements see Table 2.)

Habitat: Intestine.

Host: African elephant.

Quilonia brevicauda Khalil, 1922.
(Figs. 124-127.)

Description: Is based on two specimens. The female is much stouter and longer than the male, with a short tail ending bluntly. The male bursa is much smaller than in the other species. The external leafcrown consists of ten very slender leaflets bent near their termination. They do not project freely above the head. The

internal leafcrown consists of blunt and slightly projecting processes. The mouth capsule is very short and lies closer to the oral cavity than in the other species. The oral cavity is funnel-shaped, narrowing towards the mouth opening. Into the buccal capsule project three long formidable teeth, each is placed on the top of one of the three oesophageal columns. (Figs. 125.) A small oesophageal funnel is present. The male bursa is short and broad. It is ·63 mm. in length and ·52 mm. in breadth. The dorsal ray is short and broad, being 0·5 mm. in length. It divides in its lower third into two branches. Each in turn trifurcates giving three small sub-branches. The lateral sub-branch definitely comes off first. The median sub-branch is markedly short, being approximately half the length of the internal. The spicules are equal and similar having fine terminations ending ventrally.

(For measurements see Table 2.)

Habitat: Intestine.

Host: African elephant.

Quilonia ethiopica Khalil, 1922.

(Figs. 128-132.)

The description is based on eight specimens examined by Khalil, 1922. The body is straight, tapering towards either end. The external leafcrown consists of 12 leaflets (Fig. 130). They are broad, leaflike and slightly bent at the tip, and do not protrude above the head. The structure of the leaflets is the main characteristic of this species. The internal leafcrown is also composed of 12 leaflets, each of which is broad at its base and tapers to a point. It has a distinct cleft in the middle, and thus each is really two leaflets side by side (Fig. 130). There is a shallow oesophageal funnel surrounded by a chitinous rim lying on the three oesophageal columns (Fig. 130).

The mouth capsule is extremely shallow. The oral cavity is practically cylindrical and contracted in the middle. Buccal teeth are absent.

Male: The dorsal lobe of the caudal bursa is longer than the lateral lobes. The bursa is 0·8 mm. long and 0·6 mm. broad. The dorsal ray is ·67 mm. long. It bifurcates in its lower third. The terminal sub-branches are long and slender. The lateral sub-branch separates first. The median and lateral sub-branches appear to run more or less parallel. The pre-bursal papillae lie on the lateral lines 0·75 mm. from the end of the bursa. The spicules are equal and similar and each has a fine termination, bent forwards (Fig. 132). The accessory piece has a bulbous cephalic extremity.

Female: The tail tapers gradually. It has a rounded tip.

(For measurements see Table 2.)

Habitat: Intestine.

Host: African elephant.

Quilonia khalili, Neveu-Lemaire, 1928.

(Figs. 133-135.)

Only the female is known. The body is cylindrical and has a whitish colour. The corona is feebly marked, composed of only a few

leaflets. The diameter of the corona is ·05 mm. The mouth contains a large voluminous prominent tooth (Fig. 135). The oesophagus shows a slight constriction at the nerve ring. The valves at the end of the oesophagus are hardly visible. The posterior extremity of the female tapers to a fine point. The convolutions of the ovary reach to the anterior quarter of the body. (For measurements see Table 2.)

Habitat: Intestine.

Host: African elephant.

Quilonia loxodontae Neveu-Lemaire, 1928.

(Fig. 136-138.)

Description: Is based on two female specimens only. The body is cylindrical and has a whitish colour. The nature of the anterior extremity differentiates this species from all others. The external corona is well developed and possesses a relatively large number of leaflets—about 26. These leaflets are fine and delicate, and each has a small papilla at its extremity (Fig. 138). The diameter of the corona measures 0·15 mm. The buccal capsule is very short. The nerve ring is placed 0·3 mm. from the anterior extremity of the oesophagus. The posterior extremity tapers to a fine point. The convolutions of the ovaries reach to the anterior quarter of the body. (For other measurements see Table 2.)

Habitat: Intestine.

Host: African elephant.

Quilonia magna Neveu-Lemaire, 1928.

(Figs. 139-143.)

Description: Relatively large cylindrical worms of a whitish colour. The anterior end has prominent lateral papillae. The external corona is well developed, the leaflets of which project above the head. About 16 leaflets are present, each has a small papilla at its extremity as in *Q. loxodontae*. The buccal capsule is very short. (Neveu-Lemaire figures two teethlike structures at the base of the capsule, but makes no mention of it in his description.) The nerve ring is from 0·2 to 0·25 mm. from the anterior extremity of the oesophagus.

Male: Is slightly pointed anteriorly. The dorsal lobe of the caudal bursa is short and rounded. The dorsal ray is 0·55 mm. long. Its base is thick and it bifurcates at an obtuse angle in its posterior quarter. Each branch has a broad common stem which trifurcates into three equal sub-branches. The spicules are equal, curved and finely striated transversely; they are falciform at the end.

Female: Is larger than the male and pointed anteriorly and its posterior extremity has a long tapering point. The ovaries are convoluted extending into the anterior third of the body.

(For measurements see Table 2.)

Habitat: Intestine.

Host: African elephant.

SUB-FAMILY III, AMIRINAE Neveu-lemaire, 1924.

Diagnosis.—Strongylidae: Ventral rays of male bursa thin and united, preceded by a long and slender prebursal papilla. Lateral rays almost completely united forming a compact mass. Externo-dorsal and dorsal ray arise from a common stem. Each branch of dorsal ray tridigitate. Vulva close to anus, vagina long, uteri parallel.

Present genera: *Amira* Lane, 1914.
Khalilia Neveu-Lemaire, 1924.

Genus AMIRA Lane, 1914. (Amended—Khalil, 1922.)

Syn. Khalilia Neveu-Lemaire, 1924.

Generic Diagnosis: Fairly small worms with a thick cuticle. Cuticular bosses may be found anterior to the male bursa. The mouth is terminal and is surrounded by an external and internal leafcrown. There are six head-papillae, four sub-median and two sessile laterals, none of which are prominent. The mouth capsule is very short, its dorso-ventral and lateral axi are equal. The oesophagus is hourglass in shape, the nerve collar surrounding the constriction. There is an oesophageal funnel. The oesophagus has a cuticular lining.

Male: The dorsal lobe of the bursa may be enormously elongated. The pre-bursal papillae are very much elongated. The two equal spicules are of enormous length and very fine. A gubernaculum is present.

Female: The vulva lies close cephalad to the anus. The long vagina runs cephalad and divides into two parallel, cephalad running uteri provided with ovejectors. The colourless thinshelled ova are segmented.

Type-Species: A. pileata (Raill., Henry and Bauche, 1914.)
(In Indian elephant.)

Other species from elephants:—

A. sameera Khalil, 1922. (In African elephant.)

The generic diagnosis of Lane, 1914, based on his *Amira omra* was slightly amended by Khalil 1922, who added another species—*A. sameera*—to the genus.

Amira pileata Railliet, Henry and Bauche, 1914.

(Figs. 144-149.)

Syn. Cylicostomum pileatum Raill., Henry and Bauche, March, 1914.

Amira omra Lane, 1914.

Five males and six females were found. Five of the females contained mature ova. The bursae of all the males were more or less in a state of disintegration, so that no original camera-lucida drawings of the male extremities could be produced.

The mouth collar is separated from the rest of the cephalic end by a groove. The mouth capsule is circular, its dorso-ventral axis equal to its lateral axis. The capsule has roughly the shape of a flattened barrel so that its anterior and posterior diameter is less than its median diameter. The capsule is shallow and at its base there is a row of tubercles similar in structure to those observed in *M. murshida* and *M. falcifera*. Cephalic glands are fairly well developed (Fig. 145).

The leaflets of the external leafcrown—32 in number—extend slightly beyond the anterior rim of the mouth collar (Fig. 144). The internal leafcrown is also composed of 32 short leaflets forming the anterior margin of the mouth capsule. The specimens also possess cuticular elongate flattened projections at the base of the capsule (Fig. 145). The oesophagus is short and broad and its posterior extremity projects into the beginning of the chile-intestine forming three small lobes. An oesophageal funnel is present extending from the mouth capsule to the level of the nerve ring.

The male bursa is greatly elongated, mainly due to the length of the dorsal lobe. According to Khalil, 1922, the bursa is 1·9 mm. in length and 0·55 mm. in breadth. The cuticle in front of the bursa on the ventral surface is much thickened. The free edges of the lateral lobes of the bursa are folded inwards, obscuring the exact mode of ending of the lateral rays (Fig. 147). The pre-bursal papilla is very long, thin and wavy and measures 0·25 mm. in length. The ventral ray is bifurcated in its terminal half and the two branches lie close together. The anterior branch is thinner than the posterior (Fig. 148). The three lateral rays have a common origin. The postero-lateral ray branches before the other two rays. All three rays lie close to each other throughout their entire course.

The externo-dorsal ray is slender and pursues a curved course. It does not reach the edge of the bursa. The dorsal ray is enormously long. It measures 1·67 mm. and divides high up within 0·15 mm. of its origin. After a short course each primary division gives off a long and a short branch lying close to each other. The longer branch is 0·12 mm. and the shorter 0·05 mm. in length. These branches appear as offshoots from the main stem which continues a straight course parallel and close to the corresponding ray of the other branch. (All bursal measurements recorded here are those given by Khalil, 1922—for own measurements see Table 3).

The caudal end of the female tapers suddenly to a point.

Habitat: Large intestine and caecum.

Host: Indian elephant.

Amira sameera Khalil, 1922.

(Figs. 150-153.)

Syn. Khalilia sameera, Neveu-Lemaire, 1924.

Khalil, 1922, described this species on a single male specimen. In 1932 Mönnig described the female—his measurements are recorded (see Table 3). The two sets of measurements do not quite

coincide, nevertheless there seems to be a close resemblance between the worms described by Khalil, 1922, and those described by Mönnig in 1932.

Khalil's description is as follows: mouth collar is distinctly separated from the rest of the body by a shallow groove. It is regularly rounded in outline and measures 0·048 mm. in length, and 0·25 mm. in breadth. The external leafcrown consists of 36 slender leaflets, 0·065 mm. in length and tapering at their free ends. The internal leafcrown covers the top of the chitinous ring of the mouth capsule. It consists of 36 very short leaflets. The oesophagus is short and thick—bulbous at either end and constricted where the nerve collar is placed. Compared with *A. pileata* the oesophagus of *A. sameera* is longer and thinner. A large oesophageal funnel is present. It ends at the level of the nerve ring where the oesophagus is constricted. There are three chitinous plates lining the funnel as well as the rest of the oesophagus. The cavity of the funnel is 0·21 mm. in length and 0·175 mm. in maximum diameter.

Male: The bursa has a wrinkled appearance. Its edges are curved inwards. The dorsal is distinctly separated from the lateral lobes, and is not as elongated as in *A. pileata*. The bursa is 6·7 mm. long and 0·54 mm. broad. The pre-bursal papilla is thin and wavy and is 0·3 mm. in length. The ventral ray is bifid in its terminal half. The two branches lie close together. The lateral rays lie close together and their terminations are bent inwards similar to *A. pileata*. The externo-dorsal ray is long and ends very near the edge of the bursa. The dorsal ray measures 0.25 mm. It is massive and divided at the level of the origin of the externo-dorsal ray. Each of these primary divisions gives a thick short lateral branch, which almost immediately divides into two (Fig. 153). They are very short and do not reach the edge of the bursa. The main stem is longer and ends near the corresponding ray of the other side. The genital cone is sharply pointed and more massive than that of *A. pileata*. It is not marked with any cuticular thickenings. The cloacal opening is placed practically at the apex of the genital cone. The elongated spicules pursue a wavy course. Their tips are thickened. An accessory piece is present which measures 0·12 mm. in length. It is strongly curved from side to side and less so in longitudinal direction. The concavity of the latter curvature is directed caudally and dorsally. (For other measurements see Table 3.)

Mönnig, 1932, described the female as follows:—

Fairly stout worms tapering only slightly anteriorly, and more posteriorly. The mouth collar is distinctly separated from the rest of the body by a groove, and is a good deal narrower anteriorly than posteriorly. It is 0·31 mm. broad and 0·07 mm. high. The elongate external leafcrown arises from the cuticular lining of the buccal cavity and consists of 42 leaflets, 0·08 mm. long. (This number is bigger than that recorded by Khalil for the male—see Table 3.) An internal leafcrown consisting of short blunt leaflets is also figured (Fig. 150). The oesophageal funnel is typical, 0·24 mm. deep and 0·16 mm. wide. The tail is long, acute and bent dorsad.

Habitat: **Stomach.**

Host: **African elephant.**

TABLE 3.

Amira.

Host.	*A. pileata.*		*A. sameera.*	
	Indian Elephant.		African Elephant. (Khalil.)	(Mönnig.)
	♂	♀	♂	♀
Total length	12·5	13·9–19·9	10·5	11·5–12
Maximum diameter	·59		·45	·65
Diameter of capsule	·13		·165	·22
Length of capsule	·036		·05	
Diameter of head	·19			—
No. of leaflets (Ext.)	32·		36·	42·
No. of leaflets (Int.)	32·		36·	
Length of oesophagus	·52	·58	·6	·75
Maximum diameter of oesophagus	·31	·34	·25	·33
Nerve ring from ant. end	·27	·29	·33	·29
Excretory pore from ant. end	·72		·7	·45
Lateral pappillae from ant. end	·72	·86		—
Length of spicules	3·7		2·9	
Length of dorsal ray	1·34			—
Length of vagina		2·2		—
Vulva from anus		·22		·23
Anus from tail end		·43–·52		·62
Ova		·036x·072		·035x·039
Cuticular striations		—		·006–·008

(Measurements given in millimetres.)

SUBFAMILY IV, STRONGYLINAE Railliet, 1893.

Subfamily Diagnosis: Strongylidae, without a transverse ventral cervical groove; buccal capsule large and globular, subglobular, or infundibular. Duct of the dorsal oesophageal gland almost always prolonged as a ridge on the dorsal wall of the buccal capsule to open near its oral margin. Bursa copulatrix well developed and terminal. Parasites of the alimentary canal of Vertebrates.

Genus EQUINUBRIA Lane, 1914.

Generic Diagnosis: Fairly large and stout bursate nematodes. External leafcrown composed of numerous elements of two lengths. The internal leafcrown is composed of numerous small, short and stout elements. Mouth opening is roughly circular and the mouth capsule is more or less globular with a dorsal oesophageal gland discharging through a dorsal gutter. The buccal capsule bears denticles at its base.

Male: Bursa—ventral ray cleft, and lateral rays arise from a common trunk; externo-dorsal arises separately from the dorsal and almost immediately breaks up into three branches of which the first is the longest. The dorsal ray is split for almost half its length with two lateral branches arising from the common trunk immediately before it bifurcates. The spicules are equal and similar. There is no accessory piece.

Female: The vulva opens on a prominence close to the anus. The two uteri are convergent, running parallel and cephalad, and are furnished with weak ovejectors. Parasites of elephants.

Type-species: E. sipunculiformis (in Indian elephant).

Other species from elephants:—None.

Equinubria sipunculiformis (Baird, 1859), Lane, 1914.

(Figs. 154-161.)

Syn. Sclerostoma sipunculiforme Baird, 1859.

Cylicostomum sipunculiforme (Baird, 1859), Railliet, Henry and Bauche, 1914.

Description: Stout worms, the head separated from the body by a distinct " neck ". The oral aperture is circular and surrounded by an external leafcrown consisting of 168 rays of which 56 are long, while between each pair of these lie two shorter rays (Fig. 156). Internal to the bases of these is a row of bodies, oval when seen from the side and wedge-shaped when the head is cut off and viewed end-on. This is taken to represent an internal leafcrown. The oral aperture points slightly dorsad, and is surrounded by two lateral and four sub-median papillae, none of which are very prominent. The oral cavity and oral capsule are practically identical, they are cup-shaped, the dorsal wall being slightly shorter than the ventral (Fig. 154). Along the mid-dorsal line runs the fine duct of the dorsal oesophageal gland (Fig. 155). At the base of the buccal capsule up to ten teethlike structures (denticles) have been noticed. The anterior end of the oesophagus is enlarged and its posterior bulb ends in the chyle-intestine by the usual three valves.

Male: Is straight for the greater part of its length but turns abruptly dorsal just cephalad of the bursa. The dorsal lobe of the bursa is longer than the laterals. The bursa is supported by a large number of rays. The ventral rays are apposed, the lateral rays have markedly diverging points. The postero-lateral ray has a dorsal boss (Fig. 158). The externo-dorsal trifurcates, the ventral branch is the longest, the other two branches are considerably shorter and are irregular in outline. The dorsal ray shows three sub-divisions on each side marged by irregular prominences. A pair of small pre-bursal papillae is present. The spicules are strong, equal and similar (Fig. 160).

Female: Is straight except for a slight dorsad deviation of the tail. The anus lies at the bottom of a depression between the tail and a marked caudal-projecting conical prominence on which the vulva opens (Fig. 157).

(For measurements see Table 4.)

Habitat: Caecum.

Host: Indian elephant.

Discussion.

The material examined consisted of a large number of males and females. The specimens studied agreed in all characters with Lane's description, except in the length of the male. According to Lane the males average 15 mm., and the shortest male in my collection measured 23·7 mm.; the majority of males were about 27 mm. in length. The males examined by Lane were probably immature forms. The other measurements of this species, although all slightly bigger than Lane's, compare fairly well with his table of measurements (see Table 4).

Furthermore in all specimens examined I noticed denticles at the base of the buccal capsule, these structures are neither mentioned nor figured by Lane. Comparing Lane's figure of the dorsal ray of the bursa with the dorsal rays of males in my collection, I found the stem of the three divisions of the dorsal ray to be considerably longer than that given in Lane's drawing. In all the specimens examined the dorsal ray was much more elongated than that figured by Lane.

(Compare Figs. 159 and 161.)

TABLE 4.

Equinubria sipunculiformis.

Host.	Author's Measurements. Indian Elephant.		Lane's 1914.	
	♂	♀	♂	♀
Total length	23–27	24–28	15	27·5
Maximum diameter	1·2	1·4		—
Diameter of capsule	·30	·36		—
Depth of capsule	·20	·27		—
Diameter of head	·81	·81–·86		—
Length of oesoph.	2·2		2·3	
Maximum diameter of oesophagus	·54		—	
Nerve ring from ant. end	1·0		1·0	
Excretory pore from ant. end	2·7	3·0		—
Length of spicules	1·6		1·5	
Cuticular striations	·004		·004	
Length of vagina		1·8		1·7
Anus from tail end		·82		·8
Caudal papillae from tail end		·36		·35
Ova		·063x·036		·060x·035

(Measurements given in millimetres.)

Genus STRONGYLUS Muller, 1820.

Syn. Sclerostoma Rudolphi, 1809.

Generic Diagnosis: Elements of the external leafcrown numerous. Internal leafcrown usually absent. Buccal capsule cupshaped, thickwalled with an external circular ridge immediately behind its anterior border. Teeth may be present towards the base of the capsule.

Dorsal gutter is strongly developed. Bursa of the male is small. Spicules not barbed. Uterine branches opposed. Parasites of Equidae and elephants.

Sub-Genus DECRUSIA Lane, 1914. (Amended.)

Diagnosis: Mouth subterminal facing slightly dorsally. A cup-shaped buccal capsule with two sub-ventral teeth at its base, is present. There is a marked dorsal oesophageal duct running along the mid-dorsal line of the buccal capsule. External and internal leaf-crowns present, each composed of numerous elements. Dorsal ray is undivided except at its extreme tip. Spicules are equal and similar, fine pointed, and without an accessory piece. The female tail is blunt, the vulva is in the caudal third, the uteri are divergent, the caudad-running uterus turning immediately cephalad. Parasites of elephants.

Type-Species: D. additictia—In Indian elephant.

Other species from elephants—None.

Decrusia additictia Raill., Henry and Bauche, 1914.

Syn. Strongylus additictus Railliet, Henry and Bauche, 1914.

Decrusia decrusi Lane, 1914.

Decrusia additicia Raill., Henry and Bauche, 1915, and Lane, 1915.

Strongylus additicius Ihle, 1919.

Fairly stout worms tapering at both ends, and at a first glance looks like *Strongylus vulgaris*. The head is truncated. Dorsal wall of the oral cavity is slightly shorter than the ventral. (Fig. 164.) The oral aperture is surrounded by an internal corona composed of numerous short and blunt leaflets, which are somewhat masked by the more pronounced external leafcrown, the leaflets of which latter are pointed and longer than those of the internal leafcrown. (Fig. 162.) Both leafcrowns arise apparently at the same level. The dorso-oesophageal duct is very pronounced and runs along the dorsal wall of the mouth cavity as a canal bordered by scalloped marking. In the basal region of the capsule are two triangular prominences (teeth) with similar scalloped markings. There are six oesophago-intestinal valves. Cervical papillae are absent.

Male. The ventral rays of the bursa are long and stout. The externo-lateral ray is short. The externo-dorsal is also short, The dorsal ray is undivided except at its extreme tip—but this tip is subject to marked variations. (See figs. 167, a, b, and c.) In all variations the edge of the bursa between the two final branches is prolonged into a prominent point. The spicules have marked cross-striations at the base. They taper to extremely fine points. There is no accessory piece.

Female. The tail is very blunt but often has a short projecting spine. (Fig. 163.) The caudal papillae lie facing caudad 0·05 mm. from the tip. The vulva is situated in the posterior third of the body.

(For other measurements see Table 5.)

Habitat: Caecum and large intestine.

Host: Indian elephant.

Discussion: The material examined consists of 12 females which were in a very damaged state, due to the fact that they had been kept too long in the preserving medium (lacto-phenol). Males were absent. The females examined are definitely *D. additictia* and the measurements taken agree very favourably with those recorded by Lane, 1914, except for the total lengths (see comparative Table 5). Furthermore, the specimens very definitely show a double leafcrown, a feature which does not seem to have been observed either by Lane or by the other workers. The establishment of the presence of the internal leafcrown hence necessitates a slight amendment of the generic diagnosis to include two leafcrowns.

TABLE 5.

Decrusia additictia.

HOST.	Author's Measurements. Indian Elephant.	Lane, 1914.	
	♀	♂	♀
Total length	17, 20–21	14	15
Diameter of capsule	·5		—
Length of capsule	·4		—
Cuticular striations	·013	·01	
Length of oesophagus	1·8	1·9	
Maximum diameter of oesophagus	·41	·37	
Nerve ring from ant. end	1·07	1·1	
Excretory pore from ant. end	—	1·0	
Length of spicules	—	2·4	
Vulva from anus	7·5		7·3
Anus from tail end	·29		·3
Ova	·08x·036	·075–·04	

Genus CHONIANGIUM Railliet, Henry and Bauche, 1914 (amended). *Syn. Asifia* Lane, 1914.

Generic Diagnosis: Fairly stout, straight worms. The anterior extremity is, or tends to be obliquely truncate, so that the mouth is directed antero-dorsally, with an external leafcrown arising from the rim of the mouth opening. The external leafcrown is composed of a circle of similar leaflets. An internal leafcrown is absent. The mouth capsule is large and deep and may have teeth in its depth. Cuticular prominences may project into the cavity of the buccal capsule about the middle of its length. The bursa has an accessory ray projecting

from the dorsal aspect of the main stem of the lateral ray. The two spicules are equal and similar. The vulva lies close to the anus where the worm suddenly narrows. The vagina divides into two uteri running parallel towards the cephalic end. The tail is bluntly conical.

Parasites of elephants.

Type-species: C. epistomum—In Indian elephant.

Other species from elephants:—

C. magnostomum, n.sp.—In Indian elephant.

Choniangium epistomum (Piana and Stazzi, 1900) Raill., Henry and Bauche, 1914.

Syn. Sclerostomum epistomum Piana and Stazzi, 1900.
Asifia vasifa Lane, 1914.

Description: Males are shorter than the females. The oral aperture is surrounded by a corona of about 50 converging leaflets (Fig. 169). The leaflets extend very slightly above the head. Each leaflet is supported by an external cuticular flap. Lane, 1914, does not mention this. A large funnel-shaped buccal capsule follows on the oral cavity (Fig. 168). There are no teeth at the base of the capsule, but four pairs (not 5 pairs as recorded by Lane, 1914) of hemispheroidal cuticular prominences project into the oral cavity. One pair lies just posterior of its equator close to the middorsal duct (Fig. 169-1). Slightly more cephalic of these and on a more ventral plan lies a second pair (Fig. 169-2). A third pair lies just cephalad of the last pair and about the same level (Fig. 169-3). A fourth smaller pair lies anteriorly and ventrally in the anterior third of the buccal capsule (Fig. 169-4). The dorsal gutter shows a thin line (Fig. 168).

The valves of the chyle-intestine are exceptionally large and the anterior extremity of the chyle-intestine is wider than the posterior extremity of the oesophagus (Fig. 168).

Male bursa are longer dorsally than ventrally. The ventral rays are apposed, the points of the three branches of the lateral ray are divergent. An accessory lateral ray is present, which has a granular appearance (Fig. 172). The externo-dorsal ray is long and somewhat sinuous. The dorsal ray divides into three branches, each of which is bifurcate (Fig. 171). The spicules terminate in fine points. The accessory piece is wider caudad than cephalad and forms a trough containing the spicules.

(For measurements see Table 6.)

Habitat: Caecum.

Host: Indian elephant.

Choniangium magnostomum, sp. n. (Figs. 173-178).

Description: The worms belonging to this species are longer than those of the type-species. The oral aperture is surrounded by a corona of about 50 leaflets, which stand up straight and bend inwards at

their extreme tips, giving the appearance of semi-hooks. The corona extends well beyond the anterior extremity. Each leaflet is supported on its external surface by a cuticular flange (Fig. 176) which curves round on to the collar and ends at the level of the posterior fifth of the tooth. The mouth collar is more pronounced than in *C. epistomum*. The buccal capsule is long and deep, its walls remaining more or less equidistant anterior and posterior or in some specimens slightly wider posteriorly than anteriorly (Fig. 173). Cuticular prominences of the nature found in *C. epistomum* are absent. Small teethlike structures are present at the base of the capsule (Fig. 175). The dorsal oesophageal gutter is more pronounced than in *C. epistomum*.

The valves of the chyle-intestine are large and flaplike and the anterior extremity of the former is wider than the posterior end of the oesophagus (Fig. 175).

Male: The male is very similar to that of *C. epistomum*. The dorsal lobe is fairly long. The three branches of the lateral ray are divergent. The externo-lateral ray is slightly longer and sometimes thinner than the other two laterals. An accessory lateral ray is present, but is not granular (Fig. 178). The dorsal divides into three branches each of which bifurcates again. The inner bifurcation of the lateral branches has a characteristic swelling near its tip (Fig. 177). The spicules end in fine points and a saddleshaped accessory piece is present (Fig. 177).

(For measurments see Table 6.)

The characteristic form and shape of the external corona and the characteristic shape of the buccal cavity, as well as the absence of cuticular prominences in the latter, are the distinguishing features of this species.

Habitat: Intestine.

Host: Indian elephant.

Discussion: The material examined consisted of two females of *C. epistomum* and no males, and 12 specimens of *C. magnostomum*, including six males and six females.

On the constant features shown by the 12 specimens, especially in regard to coronal form and shape of buccal capsule, I propose to put these worms in the new species described above.

Ware, 1924, described a nematode taken from nodules removed from the intestinal wall of an elephant. He suggested that this nematode be admitted as an immature form of a member of the genus *Choniangium*, since it agreed very closely with the generic description of *Choniangium*. The dorsal ray of this parasite, figured by Ware, is typical of *Choniangium*, and the lateral lobe shows the fourth accessory ray and the female tail end is also very similar to that of *C. epistomum*. However, his figure of the capsule shows hardly any resemblance to that of either of the two *Choniangium*-species. The measurements also do not agree as will be seen from the table, but this may be accounted for by the fact that the parasite described by Ware, was an immature individual.

(Type-specimens have been deposited at Onderstepoort Laboratories.)

TABLE 6.

Choniangium.

HOST.	*C. epistomum.* Indian Elephant. (Lane, 1914).		*C. magnostomum.* Indian Elephant.		WARE'S *nematode.* Indian Elephant.	
	♂	♀	♂	♀	♂	♀
Total length	14	18·5	19–21·2	21–22·3	9–	9
Diameter of capsule	·35	·36	·31	·36	·16	
Length of capsule	·75	·75	1·0		—	
Cuticular striations	·004		·004		·018	
Length of oesophagus	1·5	1·55	1·8		·75	
Diameter of oesophagus	·32	·34	·4		·13	
Nerve ring from ant. end	1·25	1·30	1·4	1·5	·35	
Excretory pore from ant. end	2·2		2·5	2·6	—	
Cervical papillae from ant. end	2·0	2·3	2·2		·75	
Length of spicules	2·0		2·3		·6	
Caudal papillae from tail end	·12		·13		—	
Vulva from anus		·29		·31–·36		·23
Anus from tail end		·45		·4		·2
Length of vagina		1·2		1·2		·25
Ova		·05x·025		·075x·027		—

(Measurements given in millimetres.)

FAMILY II ANCYLOSTOMIDAE (LOOSS, 1905) LANE, 1917.

SUB-FAMILY NECATORINAE, Lane, 1917.

Sub-Family Diagnosis: Ancylostomidae: mouth directed antero-dorsally; with a sub-globular buccal capsule, the oral margin of which is provided ventrally with semi-lunar plates.

Genus BUNOSTOMUM Railliet. 1902.

Syn. Monodontus Molin, 1861, in part.

Bustomum Lane, 1917.

Generic Diagnosis: Capsuled bursate nematodes having the mouth guarded by ventral semilunes. The mouth cavity contains a dorsal tooth, being the freely projecting duct of the dorsal oesophageal gland. There is a pair of sub-ventral lancets at its base.

The dorsal and externo-dorsal rays are asymmetrical. The dorsal ray bifurcates to a varying degree, each branch being bidigitate or tridigitate. Spicules are equal. A gubernaculum absent.

The vulva in the female is situated in front of the middle of the body. Parasites of herbivores.

Type-Species: B. trigonocephalum, Rudolphi, 1808.—In sheep and cattle.

Other species from elephants:—

B. foliatum Cobbold, 1882.—In Indian elephant.

B. brevispiculum Mönnig, 1932.—In African elephant.

B. hamatum Mönnig, 1932.—In African elephant.

Bunostomum foliatum (Cobbold, 1882.) Rail., Henry and Bauche, 1914.

(Figs. 179-183.)

Syn. Strongylus foliatus Cobbold, 1882.

Uncinaria sangeri Alessandrini, 1905 (not Railliet, 1896).

Cobbold, 1882, describes this nematode as follows: Body smooth, of nearly uniform thickness, finely striated transversely. Head indistinct, truncated in front, with five small auriculate folds. Mouth slightly oblique, leading to a deep buccal capsule, armed with a few coarse teeth, succeeded by a long muscular oesophagus. Neck marked by two short conical papillae at the upper part, one on either side, and by two larger and longer tapering papillae placed lower down. (The latter are probably the cervical papillae.)

The bursa is bilobed, foliate. Rays few in number, and widely separated. Spicules long, flattened, and twisted at the base, tapering and finely pointed below at the apex. They measure 1·46 mm. The tail of the female is well marked, long and conical, with a wavy contour, directed backwards, and rapidly narrowing to form a subulate point. Anus in front, and immediately above the base of the tail. Length of the male is 13 mm. and of the female 15·2 mm.

Habitat: Stomach. (Tumours.)

Host: Indian elephant.

Bunostomum brevispiculum Mönnig, 1932.

(Figs. 184-187.)

The description is based on a single male specimen. The anterior end is flexed dorsad, and the mouth opening is situated on the dorsal side. Six papillae, four sub-median and two lateral, surround the mouth. The buccal cavity is lined with highly refractive chitin, varying in thickness in different places and thus forming a sinuous line in optical section. The buccal capsule is longer ventrally than dorsally. At the base of the capsule there is a moderately large dorsal tooth, 0·043 mm. long with a bifid tip, and two slightly smaller sub-ventral teeth. The oesophagus is provided with a small funnel lined with chitin; the whole oesophagus is club shaped. The excretory pore opens slightly behind the level of the nerve ring. There are a pair of fairly thick blunt cervical papillae placed at a short distance behind the level of the excretory pore.

The bursa is well developed and the dorsal lobe is asymmetrical. The ventral rays have a common stem which is longer than the two branches; the latter lie close together and do not reach the edge

of the bursa. The lateral rays originate from a thick stem which divides about half-way down the length of the lateral lobe. The externo-lateral ray diverges widely in a ventral direction from the other two; the medio- and postero-laterals are directed somewhat dorsad, run close together, and reach the margin of the bursa. The right externo-dorsal ray comes off very high from the dorsal stem, it is thin and runs a sinuous course into the right lateral lobe. The left externo-dorsal ray is given off just anteriorly to the bifurcation of the dorsal ray and passes into the left lateral lobe. The two branches of the dorsal ray have tridigitate tips which differ in the two rays. The specimen showed one spicule which is alate and twisted irregularly.

(For other measurements see Table 7.)

Habitat: ?.

Host: African elephant.

Bunostomum hamatum Mönnig, 1932.

(Figs. 188, 189.)

The description is also based on a single male specimen. It is smaller than *B. brevispiculum*. Its mouth capsule is similar to that of *B. brevispiculum*, with a slightly longer dorsal tooth (0·055 mm.). The cervical papillae are placed on a level between the nerve ring and excretory pore.

The male bursa has the same appearance as in *B. brevispiculum*, but differs from it in the case of the ventral rays, whose common stem is shorter than the two branches. There are two equal alate spicules, with their distal ends sharply bent dorsalwards and the extreme tips curved back in the form of small hooks.

(For other measurements see Table 7.)

Habitat: ?.

Host: African elephant.

TABLE 7.

Bunostomum.

	B. foliatum.		*B. brevispiculum.*	*B. hamatum.*
	♂	♀	♂	♂
Total length	·13	15·2	12·1	8·7
Maximum diameter	—		0·073	0·086
Cuticular striations	—		0·003	0·003
Vent. length of capsule	—		0·157	0·161
Dorso-ventral diameter of capsule	—		0·118	0·118
Length of oesophagus	—		1·02	1·08
Nerve ring from ant. end	—		0·52	0·50
Excretory pore from ant. end	—		—	0·55
Length of spicules	1·46		0·224	0·67

Genus BATHMOSTOMUM Raill. and Henry, 1909 (Amended).

Generic Diagnosis: Anterior extremity bent dorsally. Buccal capsule infundibular with two semilunar ventral cutting plates at its oral margin. The internal surface of the capsule is raised into a series of shelflike ridges. A dorsal gutter is present. Teeth in the depth of the buccal capsule are absent.

Male bursa: Dorsal rays are separate for nearly their whole extent, the externo-dorsal rays springing from the individual dorsal rays. The lateral rays have a ventral direction. Spicules are stout and equal. A gubernaculum present.

The vulva is a little in front of the middle of the body.

Parasites of elephants.

Type-species: B. sangeri—In Indian elephant.

Bathmostomum sangeri (Cobbold, 1882), Raill. and Henry, 1909.

(Figs. 190-198.)

Syn. Dochmius sangeri Cobbold, 1879.

Uncinaria sangeri (Cobbold, 1879), Railliet, 1897.

Uncinaria os-papillatum Piana and Stazzi, 1900.

Description: Fairly small worms with a characteristic dorsally bent anterior extremity. The semilunes are large and massive. Lane, 1921 states that the buccal cavity is wider than long; but in all specimens measured the buccal capsule was never wider than long, the width of the capsule was either equal or slightly shorter than its length. This measurement probably depends on the state of preservation of the specimens. The semilunes are not well pronounced. The shape of the buccal cavity is more or less sub-globular, its internal surface is raised mainly ventrally and laterally upwardly tilted shelf-like ridges. The ridges appear to be complete, and not interrupted as stated by Lane, encircling the cavity of the capsule. These ridges appear to be folds in the cuticular lining of the buccal capsule. The buccal capsule bears no teeth, but each ridge is pleated once on either side of the median ventral line, giving in lateral view the appearance of double supporting teeth, and in dorsal view of three pairs of sub-ventral teeth, as was wrongly interpreted by Lane. The oesophageal gutter opens into the buccal cavity as a conical projection, and it is not associated with a dorsal tooth, as was stated by Lane. The oesophagus is long and simple with a slight posterior swelling. Three intestino-oesophageal valves are present

Male: The bursal rays are fairly stout, the lateral and ventral rays tapering steadily. The divisions of the three lateral rays are about the same depth. The pre-bursal papilla is large. The spicules are stout and similar, ending in sharp truncated points. One male examined had spicules whose median parts were inflated (Fig. 195), but this is probably an abnormal condition or irregularity. A gubernaculum is present, the two halves of which form a semi-circle (Fig. 194).

Female: The vagina is short, the two uteri divergent. The tail has a long tapering point (see Figs. 198 and 191).

(For measurements see Table 8.)

Habitat: Large intestine.

Host: Indian elephant.

Discussion: The material in hand consists of an enormous number of males and females. A large number of specimens was examined and all showed the features characteristic of the above species. Buccal teeth being found to be absent, and a gubernaculum to be present, the specific description and also the generic diagnosis have been amended. The ridges in the internal surface-lining of the capsule being complete, the capsule is not fissured, as stated by Lane.

The measurements taken do not compare very well with Lane's but this difference may be due to the poor state of preservation of Lane's specimens.

TABLE 8.

Bathmostomum sangeri.

Host.	Indian Elepahnt.			
	Author's Measurements.		Lane's 1921.	
	♂	♀	♂	♀
Total length	15–16·6	18–22·1	15–16*a	20*a
Maximum diameter	·63		—	
Diameter of capsule	·27		—	
Length of capsule	·27		·24	
Cuticular striations	·006		·005	
Length of oesophagus	1·55	1·67	1·5	
Maximum diameter of oesophagus	·27	·29	—	
Nerve ring from ant. end	·63	·73	·5	
Excretory pore from ant. end	·81–·90	·86–1·09	·6	
Cervical papillae from ant. end	—		·66	
Caudal papillae from tail end		·16		·18
Length of spicules	·75		·47	
Vulva fron anus		8·9–11·4		9·0
Anus from tail end		·72		·6
Ova		·063x·033		·045x·030

(All measurements given in millimetres.)

* a. These total length measurements are taken from Yorke & Maplestone, 1926.

Genus GRAMMOCEPHALUS Raill. and Henry, 1910.

Generic Diagnosis: Anterior extremity bent dorsally. Buccal capsule wide, and narrowed posteriorly, the narrowing being more abrupt on the dorsal wall and accompanied by an infolding of the capsule. Oral margin has two semilunar ventro-cutting plates. Towards the base of the capsule a pair of lateral and a pair of subventral teeth are present, also a dorsal cone carrying a dorsal gutter. The intestine has a long anteriorly directed dorsal diverticulum, arising close to its union with the oesophagus.

Male Bursa: Ventral ray cleft to its base. The extreme-lateral and postero-lateral are close together. The externo-dorsal and dorsal arise from a common trunk. The dorsal ray has two limbs, each limb being bidigitate. The spicules are stout and alate. An accessory piece is absent.

Female: Vulva situated near the middle of the body; the uteri are divergent.

Parasites of elephants and rhinoceroses.

Type Species: G. clathratus, Baird, 1868. (In African elephant.)

Other species in elephants:

G. varedatus, Lane, 1921. (In Indian elephant.)
G. hybridatus, n. sp. (In Indian elephant.)

Grammocephalus clathratus (Baird, 1868.), Railliet and Henry, 1910.

(Figs. 200, 201, 203, 204.)

Syn. Sclerostoma clathratum Baird, 1868.
Strongylus clathratus Cobbold, 1882.

The description as given is taken from Lane, 1921.

The semilunes at the anterior extremity are large (Fig. 200), the fold in the dorsal wall of the oral capsule is marked; the medium dorsal tooth not particularly prominent; the apical notches in the sub-ventral teeth large, the anterior edges of these teeth face anteriorly and ventrally. The anterior edges of the lateral piece lie well posterior of the corresponding edges of the sub-ventral teeth (Fig. 200). The oesophagus is long and simple. The intestinal diverticulum measures 2·3 mm. (For other measurements see Table 9.)

Male: The bursal rays (Fig. 201) are relatively long. The dorsal rays are united from their base to the point of origin of the externo-dorsal rays, separate beyond, and their extremities are bifid; the outer branch curving, the inner inclining towards the midline (Fig. 204).

Female: The body narrows abruptly about the anus, and the first part of the tail is nearly cylindrical. (Fig. 203).

Habitat: Bile ducts.

Host: African elephant.

Grammocephalus varedatus Lane, 1921.

(Figs. 199, 202, 205.)

Syn. Strongylus clathratus Cobbold, 1882.
Sclerostomum clathratum Piana and Stazzi, 1900.
Nematode No. 1 Evans and Rennie, 1910.
Grammocephalus clathratus (Baird), *ex parte* Raill. and Henry, 1910.

The following description is taken from Lane, 1921:

The semilunes are somewhat small, the fold in the dorsal wall of the oral capsule is not markedly conspicuous. The ventral projection of the dorsal tooth is considerable, the apical notches of the sub-ventral teeth are small, and the anterior edges of these teeth face anteriorly and dorsally. The anterior edges of the lateral teeth do not lie posterior of the corresponding edges of the sub-ventral teeth (Fig. 199). The intestinal caecum measures 2·2 mm.

(For other measurements see Table 9.)

Male: The bursal rays are relatively short (Fig. 205). The branches of the dorsal ray are exceedingly short. The tail of the female is conical. (Fig. 202).

Habitat: Bile ducts.

Host: Indian elephant.

Grammocephalus hybridatus, sp. n.

(Figs. 206-221.)

The material consists of a large number of male and female specimens, from the bile ducts of the Indian elephant. Also numerous larval forms taken from nodules removed from the large intestine. Among the adult specimens from the bile ducts few larval and immature males and females were also present.

The males and females are more or less of equal length. The head is curved towards the dorsum and the mouth is directed obliquely, dorsally and anteriorly.

The semilunes are not large, somewhat intermediate between that of *G. varedatus* and *G. clathratus*. The fold in the dorsal wall of the buccal capsule is not well marked at all. In most specimens it is not visible. A pair of sub-ventral teeth are present, the apical notches of which are relatively small, and the anterior edges of these teeth face anteriorly and dorsally. The position and shape of this lancet is very similar to that of *G. varedatus* except that its ventral anterior edge is usually produced into an acute point, and is never rounded like that of *G. varedatus*. In one particular specimen the sub-ventral lancets had only one instead of two apical notches (Fig. 209) and their dorsal anterior edges approach the condition as figured for *G. varedatus* (Fig. 207). A pair of lateral teeth is also present. These, although subject to variation (Figs. 208, a, b, c,) are also very similar in shape and position, to those of *G. varedatus*, i.e. they have the anterior surface concave, so that the free apex forms an acute angle. The anterior edges of the lateral lancets also lie anterior of the corresponding edges of the sub-ventral teeth. (Compare Figs.)

The single dorsal tooth is not so conspicuous and receives the duct of the dorsal oesophageal gland. The oesophagus is long and simple. The intestinal diverticulum averages 2·09 mm.

Male: The bursa is elongate, particularly so in the larval males (see Fig. 215), and the externo-dorsal rays of the adult male are longer than those of *G. varedatus* and *G. clathratus* (Fig. 219). The general appearance of the bursa is very similar to that of *G. clathratus*. The structure of the larval male spicule is very different to that of the adult male spicule (compare Figs. 217 and 218).

Female: Is thickest at the vulva and tapers towards both ends. The tail ends in a long conical point, which is more acute in the larval females than in adult females. Also the tail ending points slightly dorsad in all adult females, but is straighter in larval females (compare Figs. 210, 212, 213). Rectal glands were observed in a number of females.

(For measurements see Table 9.)

Habitat: Adults in bile ducts and larvae in nodules on large intestine.

Host: Indian elephant.

Discussion: From the above description and figures presented it will be noted that the cephalic end of this nematode from the Indian elephant bears a remarkable resemblance to the cephalic end of *G. varedatus*, (from Indian elephant) whereas the male bursa closely resembles that of *G. clathratus* (from African elephant). However, the features of the cephalic extremity and the male bursa respectively, are not absolutely identical. For example, the lateral and sub-ventral lancets of the species described above are not quite identical with those of *G. varedatus*. *G. hybridatus* also differs in the almost complete absence of the fold in the dorsal wall of the capsule, and in structure and position of the oral plates. In the male bursa, the individual rays of this species are also somewhat more elongate than those of *G. clathratus*, as figured by Lane, 1921. Also the female tail end, although very similar to that of *G. varedatus*, is not identical with it as regards shape. Furthermore, the principal measurements of this worm differ widely from those recorded for the other two species (see table). Hence, according to the peculiar combination of characters shown by the anterior and the posterior end, as well as the different measurements of this worm, it is not possible to consider it as a variation of either *G. clathratus* or *G. varedatus*. Also, since the nematode parasites of the Indian and African elephant have so far been found to be quite specific to their host, the worm in question cannot be regarded as a variation of *G. clathratus* from the African elephant.

Neveu-Lemaire, 1924, described a *Grammocephalus*—species (*G. intermedius*) from the large intestine of the African rhinoceros, but according to his description (based on a few badly damaged specimens) and his figures, this species is more closely related to *G. clathratus* of the African elephant, than to the species described above; which is what one would expect, since both African elephant and rhinoceros inhabit more or less the same locality.

Since the characters of the buccal capsule and bursa, and the measurements as given above for the bile-duct-nematode under discussion, were found to be very constant in the large number of

specimens examined, including immature and larval forms, it is in my opinion justifiable to consider it as a new species, for which the name *G. hybridatus* is being proposed.

(Type-specimens have been deposited at Onderstepoort.)

TABLE 9.

Grammocephalus.

HOST.	*G. clathratus.* African Elephant.		*G. hybridatus.* Indian Elephant.		*G. varedatus.* Indian Elephant.	
	♂	♀	♂	♀	♂	♀
Total length	45·5	36	37	37	55	47
Maximum diameter	1·15	1·03	1·4	1·0	1·35	1·1
Length of buccal capsule	·46		·50	·46	·55	
Cuticular striations	·014		·012		·014	
Length of oesophagus	3·6		3·64	3·7	3·6	
Nerve ring from ant. end	1·2		1·09	1·0	1·16	
Excretory pore from ant. end	1·7		1·29	1·07	1·5	
Cervical papillae from ant. end	1·7		1·15		1·7	
Length of spicules	1·2		1·54		1·35	
Vulva from anus		17		16·7		22
Anus from tail end		·67		·80		·8
Ova		·05x·035		·058–·063x·029–·036		·068x·037

(Measurements given in millimetres.)

FAMILY III SYNGAMIDAE LEIPER, 1912.

Family Diagnosis : Strongyloidea, with a well developed chitinous subglobular buccal capsule, the oral margin of which is not supplied with leafcrowns or other cutting organs, but is thickened to form a prominent chitinous rim, and bears teeth at its base.

Parasites of the respiratory system.

Genus SYNGAMUS, v. Siebold, 1836.

Syn. Cyathostoma Blanchard, 1849.

Generic Diagnosis : Nematodes with a well developed buccal capsule provided at its surface with a large chitinous ring, and in its depth with a variable number of small triangular teeth. Two lateral and four sub-median head papillae. The males are much smaller than the females to which they are permanently coupled.

Male : Bursa short; with the following formula: ventral ray cleft, postero-lateral arising separately from the other laterals, but lying close to them; medio-lateral and antero-lateral lying close together and parallel; externo-dorsal arises separately from the dorsal; dorsal bifurcated for about half its length or more, each branch usually bi- or tri-digitate; spicules small and equal, highly chitinised, usually fairly stout, and short; gubernaculum absent.

Female: Posterior extremity conical; vulva in the anterior part of the body; uteri parallel. Oviparous, ova of very characteristic shape, slightly flattened on one side.

Parasites of the respiratory tract of birds and mammals.

Syngamus indicus Mönnig, 1932.

(Figs. 222-225.)

Description: The worms are blood-red in colour when fresh, and are often found *in copula*. The body of the male gradually increases in thickness backwards, and is constricted immediately anterior to the bursa. The body of the female also grows thicker up to a short distance behind the vulva, then gradually attenuates and is again thicker in the anal region. There is practically no mouth collar. The mouth opening is surrounded by two lateral and four sub-median papillae, and opens into a strong buccal capsule more or less subglobular in appearance which is 0·4 mm. deep and 0·44 mm. broad, including the walls in both sexes. The wall bears internally six longitudinal ridges, two lateral and four submedian, like in *S. laryngeus* Raill., 1899, and *S. hippopotami* Gedoelst, 1914. In the base of the buccal capsule there are eight teeth, 0·14 mm. high, all except the dorsal and the ventral standing against the longitudinal ridges. The cervical papillae are short and thick being at the same level as the nerve ring, or slightly posterior to it. The excretory pore is either slightly anterior to the nerve ring or level with it.

The oesophagus is clubshaped, its anterior half is narrow, and posterior it becomes thicker and reaches double the width of the anterior portion.

Bursa: The bursa in the male is very short, but otherwise well developed. The ventral rays are thick, close together and each ends with a narrow point (Fig. 224). The antero- and medio-lateral rays arise from a common stem and lie close together, while the postero-lateral arises separately, beginning sometimes with a narrow base and diverges from the other laterals. The externo-dorsal rays arise quite independently (Fig. 223); they are relatively thin rays, rather short and may end either bluntly or in narrow points like the ventrals. The two branches of the dorsal ray arise separately and their bases are quite a distance apart; they both end as if broken off with irregular, fringed extremities.

The spicules are like small nails in appearance.

Female: The female has a short tail which narrows down abruptly behind the anus. The terminal portion of the tail is bent towards the ventral side. The vagina is short and soon bifurcates and forms the two uteri. In younger specimens the uteri pass directly towards the portion of the body posterior to the vulva, while in gravid specimens the uteri describe a short loop anterior to the vulva before turning posteriorly. In gravid females the genitalia occupy a little less than three-quarters of the postvulval body portion, but in younger females they occupy nearly a half of the postvulval region of the

body. The ovarian coils are confined to the post-uterine region. Between the dense coils of the ovary and the uteri the winding oviduct can be distinguished.

The shell of the egg is thickened at the poles.

The measurements recorded by Mönnig, 1932, and Bhalerao, 1935, for this species are not quite identical. (See Tables 10 and 11.)

Habitat: Pharynx.

Host: Indian elephant.

TABLE 10.

S. indicus. Mönnig 1932.

Host.	Indian Elephant.	
	♂	♀
Total length	8·5	30
Maximum diameter	·59	·91
Cuticular striations	·026–	·045
Cervical papillae—Ant. end	1·3	1·83
Excretory pore—Ant. end	1·08	1·61
Length of oesophagus	1·34	1·92
Maximum diameter of oesophagus	·2	·44
Nerve ring—Ant. end	1·0	1·28
Length of tail	—	·338
Vulva—Ant. end	—	9·0
Ova	—	·094–·105x·049–·056

TABLE 11.

S. indicus. Bhalerao 1935.

Host.	Indian Elephant.	
	♂	♀
Total length	3·75–6·5	8·6–18·5
Maximum diameter	0·31–0·44	·335–·64
Cuticular striations	0·014	·008–·022
Cervical papillae—Ant. end	·675–1·11	·87
Excretory pore—Ant. end	·575–1·07	·77– ·95
Length of oesophagus	·9 –1·2	1·07–1·78
Maximum diameter of oesophagus	·181–·265	·22– ·33
Buccal capsule	·235–·32x·25–·32	·31–·4x·25–·35
Nerve ring—Ant. end	·66 –·76	·68 –·8
Length of tail	—	·168–·22
Vulva—Ant. end	—	4·4 –5·8
Ova	–	·088–·092x·046–·047
Length of bursa	·28–·43x·125–·18	—
Length of spicules	·067–·075	—

SUPERFAMILY OXYUROIDEA, Railliet, 1916.

FAMILY ATRACTIDAE, Travassos, 1919.

Sub-family Atractinae, Railliet, 1917.

Subfamily Diagnosis: Small worms, mouth variable, intestine simple without diverticula. Male without preanal suckers; two unequal spicules, and a gubernaculum. Female tail pointed: genitalia single, vulva situated posteriorly. Viviparous.

Genus Leiperenia, Khalil, 1922.

Generic Diagnosis: Small nematodes just visible to the naked eye. The males are slightly smaller than the females. Mouth is surrounded by more than six lips; cervical alae present; vestibule absent; anterior portion of the oesophagus is shorter than the posterior, which is slightly swollen posteriorly.

Male: Tail is incurved, long and pointed; caudal alae are absent; four postanal papille; spicules unequal; gubernaculum present.

Female: Tail long and pointed; vulva near anus. Viviparous. Parasites of elephants.

Type-species: L. leiperi, Khalil, 1922. Host: African elephant.

Other species from elephants: *L. galebi*, Khalil, 1922. Host: Indian Elephant.

Leiperenia leiperi, Khalil, 1922.

(Figs. 226-229.)

This species was described by Khalil from material which consisted of two males and three females.

Very small nematodes, females slightly longer than the males. The body narrows slightly towards the head end which is truncated in appearance. Posterior to the head cuticular expansions or cervical alae about 0·3 mm. long and 0·037 mm. in maximum breadth, are present. The mouth collar is very short and has a rounded shape. It is 0·012 mm. long and 0·06 mm. in diameter. The mouth opening is practically circular and is surrounded by ten small lips. The lip in the mid-ventral line and that in the mid-dorsal line are the largest and broadest. The lips placed on either side of these lines are smaller and rounded. There is no mouth capsule. The pharynx is a short muscular canal, demarcated from the oesophagus by a deep groove. It is 0·083 mm. long and 0·06 mm. in maximum diameter. From its cephalic end project about eight conical processes (Fig. 227) which are muscular processes from the pharynx. The oesophagus is long and thin sometimes pursuing a wavy course. Its posterior end is a little swollen, but it has the pronounced bulbar swelling which is characteristic of most Oxyuroidea. The chyle intestine is simple; near its commencement its wall is surrounded by a clear refractile band, the nature of which is unknown. A large excretory vesicle is present, situated on a raised papilla, and situated radially round the pore.

Male: The caudal end of the male is bent towards the ventral surface; its end being more rounded than in the female. About 0·16 mm. from the tip of the tail there are four papillae, two near the mid-ventral line and two on either side of the tail. The papillae are simple and project very little above the surface. The irregularly convoluted testis reaches within 0·8 mm. from the head. There are two unequal spicules, and an accessory piece.

Female: Practically straight, its tail is long and gradually attenuated to a fine point. There are four papillae on either side of the anus. There is only one ovary and one uterus, placed in the axis of the body. The vagina is short and opens in the posterior part of the body. The females are viviparous, the embryos reaching an advanced stage of development while still in the uterus.

(For other measurements see Table 12.)

Habitat: Intestine.

Host: African Elephant.

Leiperenia galebi Khalil, 1922.

(Figs. 230-232.)

Khalil describes this species as follows: Very small nematodes. The head end is truncated, while the caudal is attenuated to a fine end, more delicate in the female than in the male.

The cervical alae are 0·023 mm. broad and 0·6 mm. long. The cuticle is striated throughout its length, except for a short distance at the cephalic extremity. The mouth collar is distinct and measures 0·18 mm. in length and 0·08 mm. in diameter. The oral opening is circular and surrounded by ten rounded lips. The one in the mid-ventral line and also that in the mid-dorsal line are broader and divided into two by a superficial depression. There is no buccal capsule. The pharynx is much broader and longer than in the type-species. At its cephalic end it gives rise to eight cone-shaped processes that surround the mouth opening. The chyle intestine is simple and pursues an almost straight course. The excretory pore is placed on a large raised papilla.

Male: The spicules are unequal and an accessory piece is present which has a characteristic thick bulbous end. The tail is gracefully curved and carries a group of four papillae, 0·17 mm. from the tail end, and similar to those in *L. leiperi*.

Female: Tail short and conical unlike that of *L. leiperi*. Its tip is pointed. The anal opening is not surrounded by prominent lips, and carries four papillae on either side.

The female is viviparous. The convolutions of the single ovary reach within 0·52 mm. of the head end.

(For other measurements see Table 12.)

Habitat: Intestine.

Host: Indian Elephant.

The two species of *Leiperenia* differ mainly in the length of the spicules and accessory piece, and particularly in the shape of the female tail, which is very long and narrow in *L. leiperi*, and stumpy and broad in *L. galebi*.

TABLE 12.

Leiperenia.

Species.	*L. leiperi.*		*L. galebi.*	
Host.	African Elephant.		Indian Elephant.	
	♂	♀	♂	♀
Total length	3·8	3·9	3·25	3·8
Maximum diameter of body	·2	·21	·17	·18
Cuticular striations	·007		·008	
Length of pharynx	·083		·14	
Length of oesophagus	·38		0·39	·40
Maximum diameter of oesophagus	·073		·06	·07
Excretory pore—Ant. end	·92		1·2	
Nerve ring—Ant. end	·22		·25	
Vulva—Anus	—	0·1	—	·06
Length of tail	·38	·7	·43	·53
Longer spicule	·3	—	·25	—
Shorter spicule	·19	—	·13	—
Length of gubern	·09	—	·13	—
Embryos	—	·52x·06	—	·48x ?

SUPERFAMILY ASCAROIDEA (Raill. and Henry, 1912),

FAMILY ASCARIDAE; BAIRD, 1853.

SUB-FAMILY ASCARINAE, RAILLIET AND HENRY, 1912.

Genus TOXOCARA, Stiles, 1905, Travassos, 1913.

Syn. *Belascaris* Leiper, 1907.

Toxocara lonchoptera (Dies; 1851) (Leiper, 1911), Yorke and Maplestone, 1926.

Syn. *Strongylus elephanti* Rudolph, 1819.

Ascaris lonchoptera Diesing, 1851.

Belascaris lonchoptera Leiper, 1911, Khalil, 1922.

No description can be given of this species since no material was available, and it was described by Leiper, 1907, in unpublished notes to which no access could be had.

T. lonchoptera was the first Helminth parasite recorded from the elephant, and so far the male of this species remains undescribed.

Habitat: Bileducts.

Host: Indian elephant,

SUPERFAMILY SPIRUROIDEA, Railliet and Bauche, 1915.

FAMILY ACUARIDAE, Seurat, 1913.

Sub-family Acuariinae, Raill., Henry and Sisoff, 1912.

Diagnosis: Acuaridae, usually with two large simple lateral lips. The anterior part of the body is provided with cutaneous cordons. A long cylindrical vestibule is present; the oesophagus is cylindrical, divided into two parts; the cervical papillae are usually behind the nerve collar. Lateral cuticular flanges are usually absent.

Male: Caudal alae present, with four pairs of long, pedunculated preanal papillae; spicules unequal and quite dissimilar.

Female: Usually with a short muscular ovejector and a short vagina.

Genus *Parabronema* Baylis, 1921.

Generic Diagnosis: Polymyarian worms, having the mouth bordered by paired lateral lips, each with three papillae. The cephalic extremity is provided with dorsal and ventral cuticular shields and is ornamented with six horseshoe-shaped cordons or auricular appendages of which two are lateral, two subventral and two subdorsal. Lateral flanges are absent. The cervical papillae are a short distance behind the nerve ring. The oral aperture is of greatest diameter dorsoventrally; and passes into a long cylindrical vestibule with thick walls. The oesophagus consists of two portions, both muscular, the anterior part is short and narrow and the posterior part longer and broad.

Male: Posterior extremity spirally coiled ventrally with interrupted longitudinal ridges on the ventral surface posteriorly; small caudal alae present; four pairs of preanal, and two pairs of postanal papillae arranged somewhat asymmetrically, and in addition an extra double papilla immediately in front of the cloaca; spicules very dissimilar. A somewhat triangular accessory piece is present.

Female: The female is considerably larger than the male. The posterior extremity is curved dorsally, and the tail is short. The vulva is in the region of the termination of the oesophagus. The female is viviparous. Parasites of the stomach wall of elephants and camels.

Type-species: P. indicum, Baylis, 1921. Host—Indian elephant.

Other species from elephants:—

P. smithii Cobbold, 1882. Host—Indian elephant.

P. africanum Baylis, 1921. Host—African elephant.

P. rhodesiense Yorke and Maplestone, 1926. Host—African elephant.

Parabronema indicum Baylis, 1921.

(Figs. 233-236.)

The material examined consisted of three females and one male. Rather small worms. The female is much larger than the male. The head is conical and distinctly narrower in front than behind the auricular appendages which are horseshoe-shaped and bear a narrow groove on their free edges. These horseshoe-shaped cordons vary in shape and size (compare Figs. 233 and 235) but usually have a more or less rounded posterior margin. The oral aperture passes into a long cuticular tube which leads into the oesophagus. A thick nerve collar surrounds the cephalic portion of the oesophagus. The excretory pore is anterior to the cervical papillae more or less in the region of the nerve collar.

Female: The position of the vulva varies from 0·24-0·56 mm. caudad to the posterior end of the oesophagus. The vagina is about 0·2 mm. in length and forms a characteristic U-shaped bend before it divides into the two uterine branches which run posteriorly. The one branch runs posterior to a point about 0·32 mm. from the anus where it turns anterior again. The other branch turns anterior a short distance from its origin, and runs up to about the posterior end of the oesophagus where it forms a loop and runs once more posterior. The tail is inclined dorsally and carries a small pair of caudal papillae close to the tip. These papillae were not observed in all the specimens.

*Male:*The anterior pair of postanal papillae in the male overlap in such a way that the termination of the right papilla is well to the left of the midventral line (Fig. 236). The posterior pair of postanal papillae are symmetrically placed opposite each other. The spicules are very unequal; the left spicule is very slender and about two and a half times as long as the right. An accessory piece of irregular shape is present.

(For other measurements see Table 13.)

Habitat: Stomach.

Host: Indian elephant.

Parabronema smithii (Cobbold, 1882), Baylis, 1921.

(Figs. 237-243.)

Syn. Spiroptera smithii Raill., Henry and Bauche, 1914.

Filaria smithii Cobbold, 1882.

The material examined consisted of four males and about fifty females. Very small nematodes, the female is considerably larger than the male. The female is inclined to be curved, the tail directed dorsally. The tail of the male is spirally coiled ventrally. The auricular appendages are open towards the head end like in *P. indicum*. Their posterior margins are somewhat flat in some specimens but in others are round like in *P. indicum*. The cervical papillae are irregularly placed in the regions of the nerve collar. The excretory pore could only be made out in the female.

Female: The position of the vulva varies from about 0·06 mm. anterior to about 0·45 mm. caudad to the posterior end of the oesophagus. (Compare Figs. 241 and 242.) The vagina forms a curious U-shaped bend at a variable distance from the vulva. The posterior turn of the one uterus is from 0·40-0·48 mm. from the anus. A small pair of papillae are also present on the female tail, about 0·04 mm. from its tip. The uteri are packed with embryos, and no ova are present.

Male: The testis runs anterior to a point about 0·35 mm. from the posterior end of the oesophagus, and then turns posterior. The spicules are very unequal, and a more or less triangular accessory piece is present (Fig. 243).

(For other measurements see Table 13.)

Habitat: Coats of stomach and intestine.

Host: Indian elephant.

There are no essential differences in structure between the two *Parabronema* species described above. The two species are differentiated by differences in their respective sizes and the measurements of their various organs.

Parabronema africanum Baylis, 1921.

(Figs. 244-246.)

Syn. Sclerostomum clathratum Baird, 1868.

This species is described by Baylis as follows: A much larger worm than either *P. indicum* or *P. smithii*, and the unequality in the size between the sexes are less marked, the male attaining about two-thirds of the length of the female.

The cuticle especially near the head shows minute longitudinal striations, in addition to the usual transverse striations. The head is almost invariably bent towards the dorsal side and may be roughly described as bullet shaped. It is nearly as wide at the level of the outer papillae as behind the auricular appendages. The latter are somewhat elongate with the edges curled inwards so as to form a nearly V-shaped appendage (Fig. 244), and their " grooves " are wide and are carried on the inner surface. The tail of the female is conical with a rounded tip. Caudal papillae have not been observed. The vulva is situated either just behind or just in front of the posterior end of the oesophagus. The U-shaped bend of the vagina occurs at about 1·0-1·2 mm. from the vulva. The course of the uterine branches is very similar to that described for *P. indicum* and *P. smithii.*

Male: The caudal papillae of the male are distinctly asymmetrical, the group of four preanal papillae on the left side being much more widely separated than those on the right, so that the most posterior of the group lies at the level of the cloaca. The two terminations of the median preanal papilla appear to be situated close

TABLE 13.

Parabronema.

Species.	*P. indicum.*		*P. smithii.*		*P. africanum.*		*P. rhodesiense.*	
Host.	Indian Elephant.		Indian Elephant.		African Elephant.		African Elephant.	
	♂	♀	♂	♀	♂	♀	♂	♀
Total length	9·0	13·0	6·5	6·6–9·3	40·0	57·0	7–8	9–10
Maximum diameter	·24	0·30	·22	·24	·8	1·0	—	—
Posterior diameter of head	0·12	0·14		·09	·27	·32	—	—
Length of buccal tube	·13	·16	—	0·10	·35	·4	·15	·175
Posterior oesophagus to anterior excretory	2·5	2·8	1·5	1·5–1·9	6·5	7·2	—	—
Nerve ring—Anterior excretory	·32	·32	·22–·24	·25	·7	·83	—	—
Cervical papillae—Anterior end	·38	·38–·43	·24–·27	·27	?	?	—	—
Excretory pore—Anterior end	—	·34	?	·32	?	0·9	—	—
Vulva—Posterior oesophagus	—	·24–·56 post	—	·06 ant. to ·45 post.	—	0·1 ant. to 0·2 post.	—	·86–·9 post
Anus—Tip of tail	·20	·33	·20	·28–·35	·85	·52	—	·20
Caudal papillae—Tip of tail	—	·042	—	0·04	—	—	—	—
Cuticular striations	·006	·004	·004	·003	·01	—	—	—
Long spicule	·95	—	·63	—	3·15	—	6·12	—
Short spicule	·40	—	·23	—	·68	—	3·3	—
Maximum length of Accessory piece	·05	—	·03	—	·08	—	—	—

together near the middle line (Fig. 246). The left spicule is more than four times as long as the right. A triangular gubernaculum is present.

(For other measurements see Table 13.)

Habitat: Stomach.

Host: African elephant.

Parabronema rhodesiense, Yorke and Maplestone, 1926.

(Figs. 247-249.)

Description: The difference in total length between male and female is less marked than in the other *Parabronema* species. The distance of the posterior end of the cordons from the anterior extremity varies from 0·11 mm. in the male to 0·13-0·14 mm. in the female; the cordons are longer than in the other species. The length of the oesophagus is 1·03 mm. in the male and 1·23 mm. in the female.

No material of this species was available for investigation.

(For other measurements see Table 13.)

Habitat: ?.

Host: African elephant.

Discussion: Khalil, 1922, erroneously places the genus *Parabronema* in the family SPIRURIDAE, Oerley, 1885, some of the diagnostic features of which are the following:—

Mouth usually with trilobed lateral lips. The cervical papillae, usually at least one, in front of the nerve collar. The caudal alae in the male are well developed. The vulva in the female is usually situated near the middle of the body. Oviparous.

None of the above-mentioned features, however, are applicable to the genus *Parabronema.*

CLASS TREMATODA.

GROUP AMPHISTOMATA RUDOLPHI, 1801, e.p., NITSCH, 1919.

Definition:—Digenea: Two suckers, the anterior surrounding the mouth and the posterior terminal or ventro-terminal behind the genitalia; gut forked; excretory pore opening dorsally towards the hinder end; testes generally in front of the ovary; almost always thick worms more or less circular in section.

FAMILY PARAMPHISTOMIDAE, FISCHOEDER, 1901.

Definition:—Amphistomata: Body not divided into two portions. Ventral pouch absent.

SUBFAMILY I, CLADORCHINAE, Fischoeder, 1901.

Definition.—Paramphistomidae: Oral sucker with a pair of oral diverticula.

Genus PSEUDODISCUS, Sonsino, 1895.

Syn. Watsonius, Stiles and Goldberger, 1910.

Diagnosis: Body sub-ellipsoidal, acetabulum strong and subterminal. Oral sucker with diverticula well developed and muscular. Caeca sinous, extending to the acetabular zone. Genital pore without sucker; bifurcal. Cirrus sac absent. Vesicula seminis well developed and convoluted. Testes lobate, post equatorial, in the same zone but in separate fields. Laurers canal present. Ovary posterior to the testes. Uterus pre-ovarian and dorsal. Vitelline glands lateral, extending from oesophageal zone to acetabular zone. Parasites of mammals.

Type-species: P. collinsi (Cobbold, 1875).

Host: Indian elephant.

Other species in elephant:—

P. hawkesii. Host Indian elephant.

Pseudodiscus collinsi (Cobbold, 1875).

Syn. Amphistoma collinsi, Sonsino, 1895.
Amphistomum collinsii, Cobbold, 1875.
Amphistomum collinsii, Fischoeder, 1903.
Pseudodiscus stanleyii, Stiles and Goldberger, 1910.
Pseudodiscus collinsi, Stiles and Goldberger, 1910.

Description: The material studied consists of numerous specimens. Dorso-ventral (sagittal) and transverse serial sections were studied, as also the en toto specimens.

The measurements obtained confirm those recorded by Bhalerao, 1933.

The body is oval in shape, convex dorsally and flattened on the ventral surface. In larger specimens the ventral surface is concave. The anterior extremity is bluntly pointed and may bear small conical papillae. The posterior extremity is round, almost semi-circular and the lateral margins are convex.

The mouth is situated anterior, and is surrounded by an oral sucker constricted in the middle to form a globular oral portion and a bulbous posterior portion. The latter is bilobed and each lobe communicates posteriorly with a diverticulum measuring 0·1—1·5 mm. × 0·7—1·25 mm. The oesophagus starts from the base, between the two bulbs of the oral sucker, and continues posteriorly in a straight course. It bifurcates into two intestinal caeca which run posteriorly and end somewhat posterior to the middle of the posterior sucker. Each caecum makes an inward bend at the level of the testes.

The excretory bladder lies on the dorsal surface of the posterior sucker and the excretory pore opens in the central line behind the posterior sucker.

The testes are two large deeply lobed bodies, situated slightly posterior to the centre of the body, but their position is subject to variation and may sometimes be slightly anterior. The vasa efferentia

arise from the centre of the testis and run anteriorly for a short distance and then unite centrally to form the vesicula seminalis, a thin-walled moderately dilated, but much coiled duct. The seminal vesicle passes into a less coiled, but, relatively thicker muscular duct called the pars musculosa. The latter passes into a short narrow duct, the pars prostatica. From its ventral aspect leads a short muscular duct ventrally, called the ductus ejaculatorius which unites with the terminal portion of the uterus, the metraterm, to form a ductus hermaphroditicus. The latter is a short duct and opens into the genital atrium which communicates ventrally with the genital pore. The ductus hermaphroditicus, the ductus ejaculatorius, metraterm and the genital atrium are enclosed in a mass of muscle fibres.

The position of the ovary is slightly variable, being sometimes situated on the left and sometimes on the right side of the median line, between the testes and posterior sucker. Sometimes the ovary appears to lie in the centre of the body, which is probably its normal position, it being deflected either to the right or the left side according to the state of contraction of the worm. The oviduct arises from the anterior aspect of the ovary and runs dorsally through the shell gland.

Laurer's canal arises from the oviduct and runs postero-dorsally. Its opening is approximately 3·0 mm. from the posterior end, and approximately 2·0 mm. anterior to the opening of the excretory duct. The vitelline glands consist of sparsely scattered follicles lying on the outer side of the intestinal caeca. The follicles usually extend posterior as far as the ends of the intestinal caeca. Anteriorly their extent varies from the level of the intestinal fork to as far as the hind ends of the diverticula, or the middle of the oesophagus. The uterus is much coiled and runs anteriorly along the dorsal surface and then coils into the metraterm.

(For measurments see Table 14.)

(Figs. 250 251.)

Habitat: Large intestine.

Host: Indian elephant.

Pseudodiscus hawkesii (Cobbold, 1875), Stiles and Goldberger, 1910.

Syn. Amphistoma hawkesi, Cobbold, 1875.
Pseudodiscus hawkesi, Sonsino, 1895.
Watsonius ornatus (Cobbold, 1882).
Amphistoma ornatum, Cobbold, 1882.
Pseudodiscus ornatus, Sonsino, 1895.
Hawkesius hawkesii, Stiles and Goldberger, 1910.
Pseudodiscus watsoni, Conyngham, 1904.
Cladorchis watsoni, Shipley, 1905.
Gastrodiscus watsoni, Verdun, 1907.
Paramphistomum watsoni, Manson, 1908.
Watsonius watsoni, Stiles and Goldberger, 1910.
Watsonius macaci, Kobayashi. 1915.

Description: The material studied consists of half a dozen specimens.

The worms are conical with the anterior end tapering and the posterior end hemispherical. The dorsal surface is convex, while the ventral is slightly concave or may tend to be flat. The genital pore lies more or less in a pit on the ventral surface, and opens on a genital papilla.

In external appearance the worms appear to be more elongate than *P. collinsi* and also the oral pole is relatively more acutely pointed.

The mouth is somewhat elliptical and directed dorso-ventrally. The oral sucker appears to be divided into an oral portion which is globular in form, and a short posterior or oesophageal portion. From the latter originate lateral suctorial pouches (diverticula) which project caudad and laterad of the point of origin of the oesophagus. The oesophagus passes into a muscular pharynx before bifurcating into the lateral intestinal caeca. The intestinal caeca pursue a wavy course posteriorly and usually end in the equatorial zone of the posterior sucker.

The testes are one behind the other and in some cases their zones are slightly overlapping. The testes are deeply lobate, almost branched. The vas efferens emerges from the dorsal aspect of each testis and unites to form the vas deferens which is much coiled in the first part of its course and passes into a coiled well-developed musculosa which in some cases much resembles a cirrus sac. The musculosa passes into a short prostatica. The terminal portion of the vas deferens is a short narrow duct, the ductus ejaculatorius, which, at the base of the genital papilla unites with the terminal portion of the uterus to form the ductus hermaphroditicus.

The ovary lies post-testicular, a little to the left of the median sagittal plane, and close to the acetabulum. The vitelline glands are composed of small follicles, which longitudinally, extend the whole length of the caeca in the extracaecal area. Their caudal portions however, may extend slightly into the intercaecal area.

Laurer's canal and an excretory vesicle are both present.

(For measurements see Table 14.)

(Figs. 252-253.)

Habitat: Colon.

Host: Indian elephant.

SUB-FAMILY II. PFENDERINAE (Fukui, 1929), as amended Bhalerao, 1935.

Definition: Paramphistomidae, with conical body. Posterior sucker papillated or not papillated. Oral sucker with paired diverticula. Oesophagus with bulb. Intestinal caeca simple, diverticulated or heteromorphic. Testes lobed, symmetrical. Cirrus sac spindle-shaped and muscular. Inside cirrus sac male genital duct differentiated into vesicula seminalis, pars prostatica and cirrus. Genital

sucker absent. Vitellaria lateral. Excretory system coiled. Lymphatic system consisting of 3 pairs of canals. Parasitic in Indian elephant.

TABLE 14.

Host.	*P. collinsi.*	*P. hawkesi.*
	Ind. elep.	Ind. elep.
Total length	5–12 mm.	3·5–6 mm.
Width	3– 7	2–3
Thickness	2–3·5	2–3
Gen. pore—Anterior end	2·5–4·5	2·1
Length of oesophagus	·9–1·5	±1·0
Excretory pore—Posterior end	·4– ·84	± ·3
Testes	1–2·5x·75–2·25	·4x·36–·39
Ovary	·40–·87x·25–·75	·5x ·8
Shell gland	·16x·18	·06
Diameter of Acetabulum	1·2–2·1	1·1–1·60
Ova	·135x·086	·135x·07

(In millimetres.)

Genus PFENDERIUS, Stiles and Goldberger, 1910.

Diagnosis: Body conical, convex dorsally, slightly convex ventrally. Ventral pouch absent. Acetabulum terminal with projecting margins. Genital pore without sucker. Excretory pore in vesicular zone, caudad of Laurer's canal. Oral sucker with one (anterior) sphincter and a pair of well developed diverticula. Testes two, lobed, fields separate, zones coincide. Cirrus sac present. Genital sucker absent. Ovary and shell gland post-testicular. Eggs operculate. Parasites of mammals.

Type-species: P. papillatus (Cobbold, 1882), in Indian elephant.

Other species in elephant:

P. birmanicus, Indian elephant.

P. heterocaeca, Indian elephant.

Pfenderius papillatus (Cobbold, 1882), Stiles and Goldberger, 1910. (Figs. 254-255.)

Description: Conical worms, 4·5-5·5 mm. in length, 2·5-2·75 mm. broad and 1·7 mm. thick, slightly bent ventrad; greatest diameter posterior, attenuating gradually and considerably cephalad. The genital pore is in the oesophageal zone, about 1·0-1·5 mm. from the oral end. The posterior sucker measures 1·7 mm. transversely and 1·4 mm. in dorso-ventral diameter. Its opening is directed slightly ventrally and its surface bears prominent papillae. The oral sucker has two caudal lateral diverticula, and has a well defined sphincter about 0·12-0·14 mm. from the oral end. The intestinal caeca pursue a wavy course and extend to the acetabular zone, and then curve slightly cephalad before they terminate.

The excretory vessel is well developed, dorsal of the cephalic half of the acetabulum.

The testes are situated in the equatorial zone, their fields are separate and their zones nearly coincide. They are lobate, and measure 0·4 mm. in diameter. The seminal vesicle is a coiled duct. A pyriform cirrus sac is present, and is 0·44 mm. long, and 0·33 mm. in greatest diameter.

The ovary is post-testicular, intercaecal, pre-acetabular and nearly or quite median. The vitellaria consist of sparsely scattered small follicles extending the length of the intestinal caeca. Laurer's canal open in the zone of the shellgland. The ova are elliptical and measure $0 \cdot 15 \times 0 \cdot 07$ mm.

Habitat: Colon.

Host: Indian elephant.

Pfenderius birmanicus, Bhalerao, 1935.

(Figs. 256-257.)

Description: The worms are oval, with the anterior end attenuated and the posterior hemispherical. The dorsal surface is convex and the ventral aspect is almost flat with a little concavity. The worms measure 2·3 mm. in length, and 2·12 mm. in breadth. The mouth is subterminal and surrounded by papillae. The acetabulum is situated at the posterior end of the body, and is subterminal, 0·92-1·02 mm. in diameter. The genital pore is situated 0·48 mm. from the anterior extremity.

The oral sucker measures $0 \cdot 39 \times 0 \cdot 46$ mm., and has two well developed diverticula. The oesophagus enlarges posteriorly into a large oesophageal bulb composed of circular muscle fibres and which is surrounded by thick glands. The intestinal caeca run laterally and terminate lateral to the acetabulum. The caeca are diverticulated internally, the diverticula being either simple or divided. The excretory bladder is pearshaped, and opens posteriorly on the dorsal side.

The testes are situated antero-lateral to the posterior sucker. They are lobed and measure 0·28-0·33 mm. × 0·31-4·46 mm. The coiled vas deferens enters a muscular, spindleshaped cirrus sac measuring $0 \cdot 5 \times 0 \cdot 3$ mm.

The ovary is situated immediately in front of the posterior sucker, and measures $0 \cdot 25 \times 0 \cdot 2$ mm. The shellgland is situated to the left of the ovary almost at the same level. Laurer's canal opens on the dorsal side slightly lateral to the central line. The uterus is coiled, and its course is similar to that of the other species of *Pfenderius.* The vitelline follicles extend laterally to the intestinal caeca and the posterior sucker. The eggs are elliptical, and measure $0 \cdot 16 \times 0 \cdot 09$ mm.

Habitat: Large intestine.

Host: Indian elephant.

Discussion: Bhalerao, 1935, classes this species under the subfamily PFENDERINAE of the family PARAMPHISTOMIDAE, on account of the elongate, spindle-shaped and muscular cirrus sac, and the oral sucker with lateral diverticula.

Pfenderius heterocaeca (Fukui, 1926), Bhalerao, 1935.

(Figs. 258-259.)

Syn. Tagumaea heterocaeca, Fukui, 1926.

Description: The worms measure 3·13-4·8 mm. in length, 2·5-3 mm. in breadth, and 1·8-2·4 mm. in thickness. The body is oval in shape and somewhat flattened dorso-ventrally. The dorsal side is convex and the ventral is either quite flat or slightly concave. The anterior end is attenuated while the posterior is hemispherical. The genital pore is ventral, situated 0·7-1·09 mm. from the anterior extremity. The posterior sucker measures 0·97-1·27 mm. in diameter, and is situated on the ventral surface of the body.

The mouth-opening lies at the bottom of the papillated anterior portion, and is directed towards the ventral aspect. The mouth is surrounded by the oral sucker which measures 0·61-0·78 × 0·50 mm. Posteriorly the oral sucker has two simple diverticula. The oral sucker has a sphincter near its anterior end. The oesophagus measures 1·53 mm. in length. At its posterior end the oesophagus has a muscular bulb which is composed of alternate layers of circular and longitudinal muscles. The intestinal caeca terminate on either side of the posterior sucker. They are short and stout and are divided into two parts; a much swollen anterior portion, and a slender posterior portion.

The testes are two large bodies lying connubially slightly in front of the acetabulum, and measuring 0·425-0·51 × 0·33-0·425 mm. They may be either bi- or tri-lobed. The vas deferens is coiled, and enters the thick, muscular, clubshaped cirrus sac, measuring 0·62-0·82 × 0·7-0·33 mm. In the cirrus sac the vas deferens forms a long clubshaped vesicula seminalis which is again followed by a short thin pars prostatica surrounded by non-cellular prostatic glands. At the terminal end of the male genital duct is the cirrus which is conical and muscular, and almost as long as the vesicula seminalis. The last portion of the genital duct enters the hermaphroditic bulb and opens on a genital papilla which is not very prominent.

The ovary is oval in shape, and measures 0·23-0·33 × 0·2-0·3 mm., and lies on the antero-lateral border of the posterior sucker, close behind the left testis. The shellgland is larger than the ovary and measures 0·33-0·42 × 0·19-0·323 mm. It is situated posterior to the ovary. Laurer's canal opens dorsally, slightly lateral to the central line. The oviduct opens together with the male genital duct on the genital papilla. The vitelline glands consist of follicles lying external to the intestinal caeca and the posterior sucker. The follicles extend the whole length of the intestinal caeca up to almost the centre of the posterior sucker. The excretory vesicle is pearshaped, and opens almost at the posterior end on the dorsal surface of the body.

The lymphatic system consists of three pairs of main canals, which communicate with one another in the region of the oral and posterior sucker.

The ova measure $0 \cdot 135 \times 0 \cdot 08$ mm.

Habitat: Intestine.

Host: Indian elephant.

Discussion: Fukui, 1926, distinguishes the genus *Tagumaea* from the genus *Pfenderius*, on the following characters:—

1. The acetabulum, which is not papillated in the former, but papillated in the latter.

2. The oesophageal bulb, which is composed of alternate bundles of circular and longitudinal muscles in the former, but of thick bundles of circular muscle only, in the latter.

3. The caeca, which is divided into an anterior broad, and posterior short and slender portion in the former, but which is undivided in the latter.

The species described above resembles *Tagumaea* in having the musculature of the oesophageal bulb composed of alternate layers of circular and longitudinal muscle fibres. It differs from both the genera however, in that the caeca are diverticulated internally. Since the species in question has also some characters in common with *Pfenderius papillatus* and *Tagumaea heterocaeca*, Bhalerao, 1935, proposes to drop the genus *Tagumaea*, and to include the species *heterocaeca* under *Pfenderius*, so that the genus *Tagumaea* then lapses into synonymy with the genus *Pfenderius*.

Although specimens of *P. heterocaeca* were not available for study, the present writer agrees with the abovementioned author that relatively insignificant characters such as the papillated or non-papillated condition of the acetabulum, the texture of the musculature of the oesophageal bulb, and the simple branched or heteromorphic nature of the intestinal caeca, do not form a valid basis for the creation of a new genus.

SUB-FAMILY III BRUMPTINAE, Stunkard, 1925.

Diagnosis: Paramphistomidae: Body sub-pyriform, with two posterior prolongations containing most of the vitellaria. The acetabulum is small, situated at the point of insertion of the auricular appendages. Ventral pouch absent. Oral sucker with diverticula. Pharynx absent. Genital sucker present. Testes lobate, their fields adjacent and zones coinciding. Ovary post-testicular. Vitellaria lateral, in the auricular prolongations of the body. Cirrus pouch present. Intestinal caeca large and sinuous. Lymphatic system complex.

Genus BRUMPTIA, Travassos, 1921.

Syn. Cladorchis, MacCallum, 1917.

Diagnosis: Body sub-pyriform with two posterior appendages in the form of an ear, and which face ventrally. Acetabulum small, situated between the two auricular formations. Oral sucker with well developed diverticula. Pharynx absent. Genital pore with a genital sucker, median, post-bifurcal. A large cirrus pouch present. Testes lobate, with zones coinciding and fields adjacent, intra-caecal, and partially in the caecal zone. Uterus dorsal. Ovary post-testicular. Vitellaria are branched, situated in the auricular appendages. The lymphatic system is complex.

Type-species: B. bicaudata, from the African elephant.

Brumptia bicaudata (Poirier, 1908), Travassos, 1934.

Syn. Amphistoma bicaudata, Poirier, 1908.
Cladorchis gigas, MacCallum, 1917.
Brumptia gigas, Travassos, 1921.
Brumptia gigas, Maplestone, 1923.
Brumptia gigas, Joyeux and Mathias, 1926.
Brumptia gigas, Fukui, 1929.

Description: The worms are 12-15 mm. in length, and 7-9 mm. in breadth. The body consists of two portions; an anterior conical portion, and a posterior part consisting of two crescentic appendages, The ventral surface is almost flat from side to side, while the dorsal surface is convex.

The acetabulum is slightly in front of the posterior extremity of the body of the worm and is situated entirely on the ventral surface, and directed ventrally. The genital pore is situated about midway between the two suckers in the midline of the ventral surface.

The most characteristic feature of the worm is the presence of two large crescentic caudal appendages which arise from the postero-lateral borders, and measure about 5 mm. in length when fully extended. As a rule their borders are incurved towards the ventral surface, so that they appear somewhat shorter and tend to overlap the posterior sucker.

The oral sucker surrounds the mouth and bears two large muscular diverticula which run in a dorsal and slightly posterior direction.

The oesophagus is simple and passes into the intestinal caeca which pursue a wavy course laterally and end in the dorsal part of the caudal appendages.

The testes are large oval organs lying side by side in the lateral fields in front of the acetabulum, and the posterior part of the cirrus pouch lies between their anterior ends. The testes are lobed and measure about 3·5 mm. in diameter. The vas deferens forms a dilated thin-walled sac, the seminal vesicle which runs into the cirrus pouch (4 mm. in diameter), and then passes into the pars prostatica which eventually unites with the uterus near the centre of the cirrus pouch. The genital duct opens on a genital papilla.

The genital pore is provided with a small sucker surrounding its opening (see Fig. 261), and the genital papilla lies within the genital sucker and is surrounded by a small atrium which would obviously disappear if the papilla were extruded. The ovary lies towards the dorsal surface between the testes and slightly to one side of the midline. The shellgland lies on the medial aspect of the ovary. Laurer's canal runs dorsally from the shellgland, and curving posteriorly over the anterior end of the excretory bladder, it opens in the midline above the middle of the bladder far in front of the excretory pore.

The vitellaria consist of numerous collections of follicles which almost entirely fill the two caudal appendages. In some cases a few follicles may lie in front of the acetabulum. The eggs are oval and operculated and measure 0·112 – 0·116 × 0·076 mm. (Figs. 260 and 261).

Habitat: Large intestine.

Host: African elephant.

SUB-FAMILY IV GASTRODISCIDAE, Stiles and Goldberger, 1910.

Definition: Paramphistomidae; with flattened leaflike bodies, divided into anterior and posterior portions. Oral sucker with paired diverticula. Cirrus pouch and genital sucker absent.

Genus GASTRODISCUS, Leuckert, 1877.

Diagnosis: Body divided by a constriction into a small, nearly cylindrical, anterior portion and a large, discoidal, ventrally concave, posterior portion. The latter is covered with regular rows of large papillae and contains the genital glands. A ventral pouch and genital sucker are absent. The posterior sucker is small and sub-terminal. The oral sucker bears a pair of diverticula. The intestinal caeca extend posterior behind the testes. The ovary is post-testicular. The vitelline glands are mainly lateral to the intestinal caeca.

Parasites of mammals.

Type-species: G. aegypticus (Cobbold, 1876).

Host: Equidae.

Gastrodiscus secundus, Looss, 1907.

(Fig. 262.)

This worm, very common in equines in India, was first recorded from elephants by Bhalerao, 1933. He describes it as follows:—

The body constructed into a cylindrical or slightly depressed cephalic portion, and a broad, oval, or disclike posterior portion. The body measures 7-8 mm. in length, and 5-8 mm. in maximum breadth. The thickness is 1·75- 2 mm. The cephalic portion is 1·87-2·6 mm. broad and 1·09-1·17 mm. long. The disc is 6·3-6·5 mm. long, and 5·8 mm. broad.

The mouth is situated at the anterior extremity on the ventral side. Tactile papillae open at a distance of 0·4 mm. from the oral aperture.

The sides of the abdominal disc tend to roll down ventrally. The ventral surface of the cephalic portion behind the mouth is slightly excavated. The ventral surface of the disc is covered with papillae or pseudo-suckers, bearing on their summits small slitlike openings.

The genital opening is situated 1·12-1·48 mm. from the anterior extremity of the disc. The genital ducts open close together separately on a genital papilla.

The posterior sucker is situated centrally at the posterior end of the body, and measures 1·2-1·7 × 0·98-1·08 mm.

The mouth is surrounded by an oral sucker measuring 0·32-0·4 × 0·46-0·65 mm. It is followed by a portion into which open the two diverticula measuring 0·54-0·63 × 0·35-0·4 mm. The oesophagus is a long straight tube measuring 1·3-1·7 × 0·23-0·25 mm. Its walls are glandular. At a distance of about 0·7 mm. from the anterior end of the disc the oesophagus bifurcates into two intestinal caeca. The latter are straight and pass along the sides of the body and end at a distance of about 1·49-1·52 mm. from the posterior end. In some cases the caeca pursue a somewhat wavy course.

The testes are situated diagonal to each other in the centre of the body. They measure 0·98-1·2 × 0·88-0·98 mm. The anterior testis is on the left side. The coiled vas deferens lies anterior to the left testis and opens on the genital papilla. The ovary also lies on the left side and measures 0·4-0·48 × 0·38-0·43 mm. The uterus takes a sinuous course between the testes and passes on the right side of the vas deferens.

The shellgland lies to the right side of the ovary and measures 0·62 × 0·36 mm. The vitelline glands extend from the level of the genital pore to the middle of the posterior sucker. The follicles lie mostly in the extra-caecal area although a few may overlap the intestinal caeca as well.

The ova measure 0·125-0·16 × 0·09-0·1 mm.

Habitat: Large intestine.

Host: Indian elephant.

GROUP DISTOMATA.

Definition: Digenea; with two suckers, the posterior of which is ventral, separated from the hind end by at least a part of the genitalia.

FAMILY FASCIOLIDAE.

Definition: Large flat worms with the genital pore in front of the ventral sucker, and with much branched testes and intestinal caeca.

Genus FASCIOLA, Linnaeus, 1758.

Diagnosis: The anterior end frequently forms a conical portion marked off by distinct " shoulders " from the rest of the body. The intestinal caeca are much branched. The ventral sucker is situated in the anterior region of the body. The ovary and testes are much branched.

A cosmopolitan parasite.

Type-species: F. hepatica, Linnaeus, 1758.

Species in elephant: F. jacksoni, Cobbold, 1869.

Fasciola jacksoni, Cobbold, 1869.

(Fig. 263.)

Syn. Cladocoelium elephantis, Diesing, 1858.
Distomum elephantis, Diesing, 1858.
Distomum jacksoni, Braun, 1892.
Fasciolopsis jacksoni, Looss, 1899.

Description according to Bhalerao, 1935: The worm is more or less pear shaped in appearance. The anterior and posterior ends have a tendency to curl down ventrally. In some cases the cephalic end is distinctly set off the rest of the body, and in such cases the " shoulders " are very well defined. In other specimens the cephalic end cannot be so well distinguished from the rest of the body.

The worms measure 12-14 mm. in length, and 9-12·5 mm. in breadth, and 1·5-2 mm. in thickness. The whole body is covered with a thick cuticle beset with close-set alternately transverse rows of spines which measure ·042-·055 mm. in length. The spines on the dorsal and ventral sides of the body are equally stout. The mouth is surrounded by an oral sucker which is elliptical in shape and measures 0·52-0·64 mm. × 0·44-0·43 mm. The pharynx is large. The oesophagus is very short. The intestine is branched both internally and externally, but the branches on the outer side are more profuse than those on the inner side.

The ventral sucker measures 1·18-1·41 × 0·97-1·017 mm. Its cavity is somewhat triangular, and in some cases a notch is seen posteriorly. The genital pore is situated about 2·5 mm. from the anterior end.

The testes lie centrally in the body, one behind the other. They occupy nearly half the area of the body. The cirrus sac is pyriform and is situated anterior to the ventral sucker. It measures 0·73 × 0·56 mm. The cirrus is a very long structure and may be coiled in the cirrus sac or may protrude considerably out of it.

The ovary is a branched structure lying on the right side of the central line, immediately anterior to the testis. It measures 1·37 × 1·28 mm. The shellgland lies centrally, anterior to the testes, and measures 1 × 0·74 mm. The receptaculum seminis is absent. Laurer's canal commences from the oviduct and opens to the

exterior on the dorsal side of the shellgland. The vitellaria are very extensive and occupy nearly two-thirds of the body. The vitelline follicles are small and are situated in the meshes of the intestinal branches. The vitelline receptacle or yolksac is situated in the centre and measures 0·14 × 0·1 mm. The uterine coils lie between the shellgland and the ventral sucker. The eggs are operculated and measure 0·110-0·120 × 0·05-0·062 mm.

Habitat: Bileducts and duodenum.

Host: Indian elephant.

CLASS CESTODA.

ORDER CYCLOPHYLLIDEA, CARUS.

Definition: Vitelline gland compact and unpaired, in neighbourhood of ovary.

FAMILY ANOPLOCEPHALIDAE, FURHMANN, 1907.

Definition: Rostellum unarmed, mature proglottides usually broader than long, testes mostly anterior or lateral to female organs.

SUBFAMILY ANOPLOCEPHALINAE, Blanchard, 1891.

Diagnosis: Anoplocephalidae: Genital ducts usually pass dorsally to longitudinal excretory vessels. Uterus persistent and tubular, sac-like, branched or reticular. Adults in mammals and birds.

Genus ANOPLOCEPHALA, Blanchard, 1848.

Diagnosis: A single set of reproductive organs in each proglottis. Genital canals pass dorsally to longitudinal excretory vessels. Genital pores unilateral (or occasionally alternating). Vaginal pore ventral to cirrus-sac. Testes aporal, or scattered uniformly throughout the proglottis. Female glands poral, but in the parasite from the elephant the female glands median in position. Uterus a transversely elongated sac with pocket-like appendages anteriorly and posteriorly. Eggs with well developed pyriform apparatus. Adults in mammals and birds.

Type-species: Anoplocephala perfoliata (Goeze, 1782).

Species in Elephant: A. manubriata, Raill., Henry and Bauche, 1914.

Anoplocephala manubriata Raill., Henry and Bauche, 1914.

(Figs. 264-266.)

Description: Railliet, Henry and Bauche, 1914, describe the worm as follows:—

Only two specimens and a number of gravid proglottides were encountered in an Indian elephant.

The two specimens presented the following measurements:—

The first 2·6 cm. long and 1·6 cm. wide, the second 1·5 cm. long and 1·2 cm. wide, but some of the isolated gravid proglottides, however, measured up to 4 cm. in width.

The scolex forms a voluminous mass, tetragonal in shape, and depressed from before backwards. It measures 6 mm. to 7 mm. transversely and from 5·5-6 mm. dorso-ventrally. The scolex carries no hooks, but it is provided with four large suckers which are separated from one another by two cross-shaped grooves of which the dorso-ventral groove is more pronounced. In the one specimen this groove was even continued onto the dorsal and ventral faces of the strobilus. There is no neck; a simple fold separates the scolex from the strobilus. The strobilus attains its maximum width about 1·5 cm. from the scolex. At the point of maximum width the strobilus forms a lateral bulge which is more marked on the left side. From this point backwards the strobilus becomes narrower but the individual segments become longer and less thick. The average thickness of the segments are from 2·5-3 mm. The anterior segments of the strobilus are very short (1·0 mm. long), but they increase in length towards the middle and again shorten towards the posterior end.

There are two longitudinal and ventral, excretory canals which are united by a transverse canal in the posterior region of each segment. The transverse canals of successive segments are also in communication by means of a well developed longitudinal anastomosis.

There are two simple lateral nerve strands. These lie external but very close to the lateral excretory canals.

The genital organs are fully developed at a distance of two cm. from the scolex, but at this point the uterus contains no eggs yet.

The testes are small and numerous; distributed in the dorsal region of the segment. The testicular zone stops 1·5 mm. from the lateral margin of each segment. The vas deferens runs the entire width of the testicular zone in the segment. In reality there are two vasa efferentia, very unequal in length, which unite after a short distance to form a much convoluted seminal vesicle. The cirrus pouch is fusiform and measures 1·5-1·8 mm. in length by 0·25-0·28 mm. in width. The ejaculatory duct forms one or two coils before leading to the exterior.

The genital pore is situated anteriorly on the left side of each segment, forming a small atrium which is directed anteriorly.

The penis was not seen in the evaginated condition, but appears to be ciliated.

The ovary almost surrounds the vitellarium and measures 1·5 mm. It occupies a considerable central portion in the posterior region of the segment.

The vagina after a short distance forms a large receptaculum seminis, situated 0·3 mm. from the genital pore. The uterus is conspicuous in the posterior segments of the strobilus and consists

of a large transverse canal with anterior and posterior pocket-like appendages, but in the gravid proglottides the uterus is no more than a transverse band consisting of small pockets containing the eggs.

The eggs are irregular in outline, circular or slightly pentagonal or hexagonal in shape, with a diameter of 0·07-0·08 mm. The eggs have a pyriform apparatus measuring 0·05 -0·055 mm. in length, and contain embryos measuring from 0·017-0·022 mm.

Habitat: Intestine.

Host: Indian elephant.

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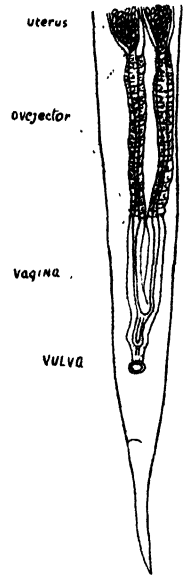

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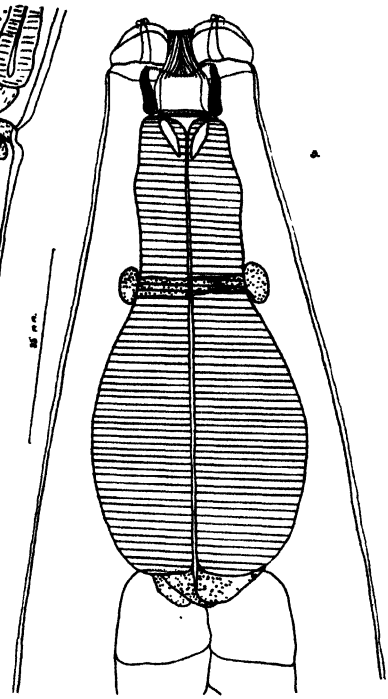
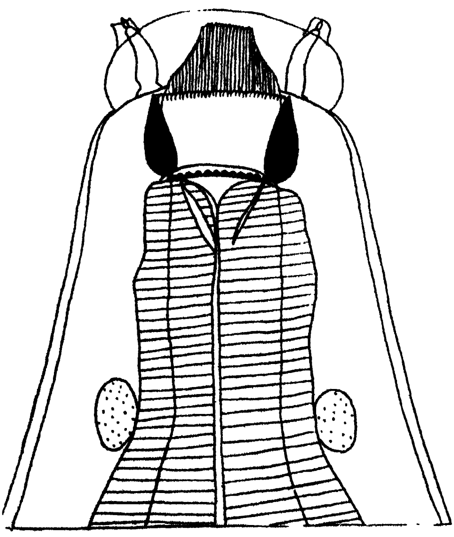


Fig. 1.—*Murshidia*—female genital ducts. (Orig.)
Fig. 2.—*Murshidia murshida*—female tail. (Orig.)
Fig. 3.—*Murshidia murshida*—anterior end, dorsal view. (Orig.)



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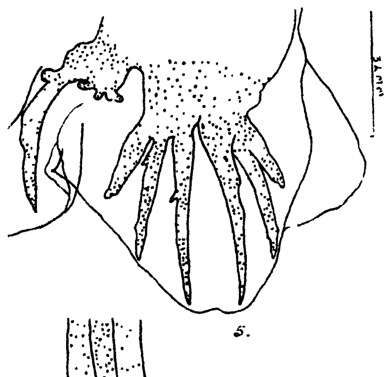

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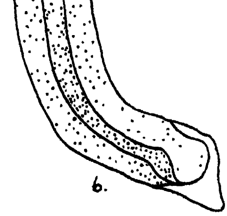

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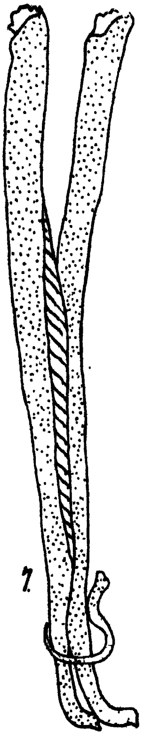
Fig. 4.—*Murshidia murshida*—anterior end, lateral view. (Orig.)
Fig. 5.—*Murshidia murshida*—male bursa, dorsal ray. (Orig.)
Fig. 6.—*Murshidia murshida*—" beaked " tip of male spicule. (Orig.)



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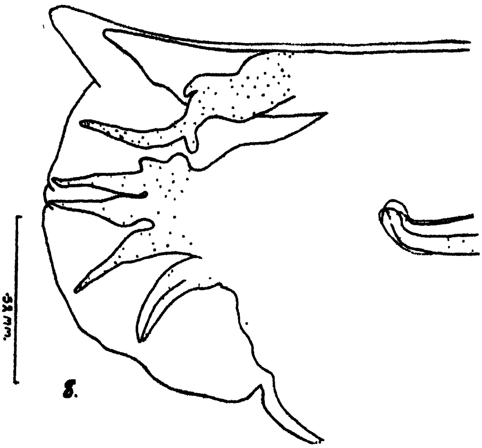

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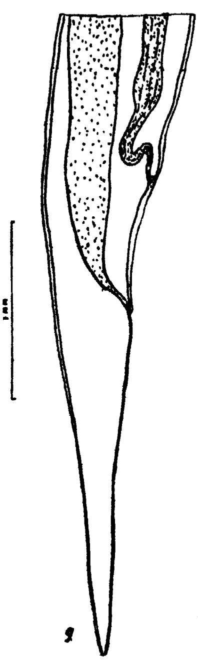
Fig. 7.—*Murshidia murshida*—spicules of male. (Orig.)

Fig. 8.—*Murshidia murshida*—male bursa, lateral view. (Orig.)



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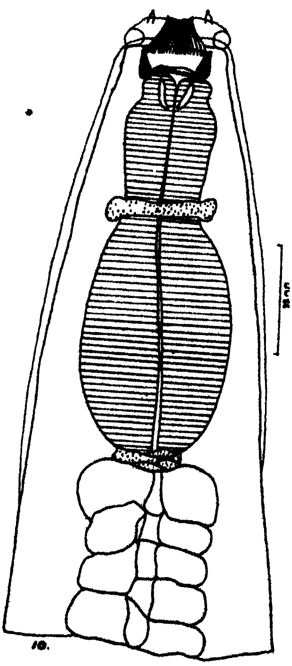

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Fig. 9.—*Murshidia falcifera*—female tail. (Orig.)
Fig. 10.—*Murshidia murshida*—anterior end, lateral view. (Orig.)

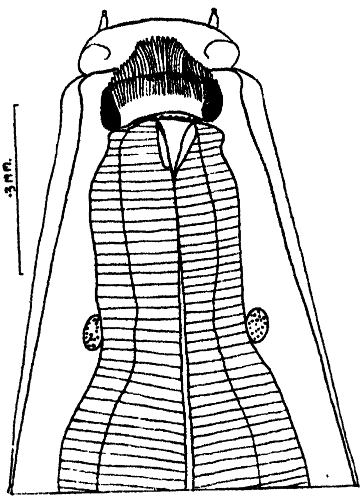


Fig. 11.—*Murshidia falcifera*—head, lateral view. (Orig.)

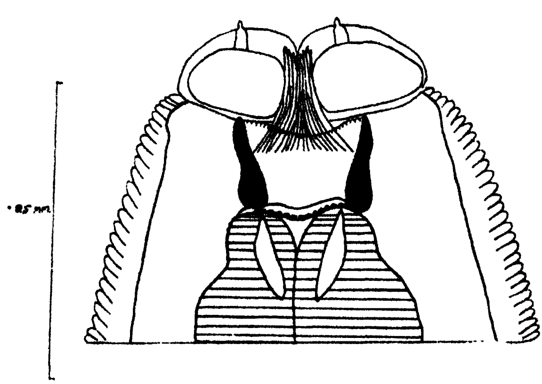


Fig. 12.—*Murshidia falcifera*—head, dorsal view. (Orig.)

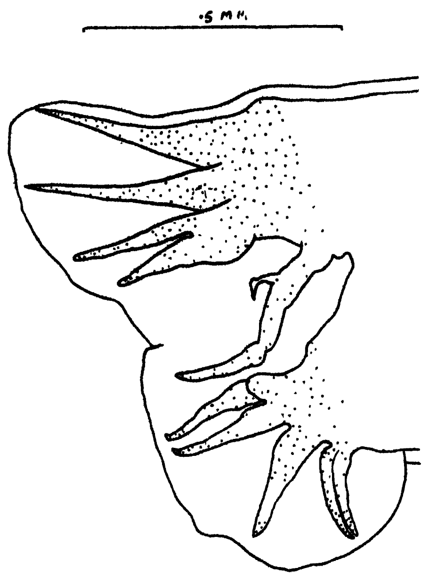


Fig. 13.—*Murshidia falcifera*—male bursa, lateral view. (Orig.)

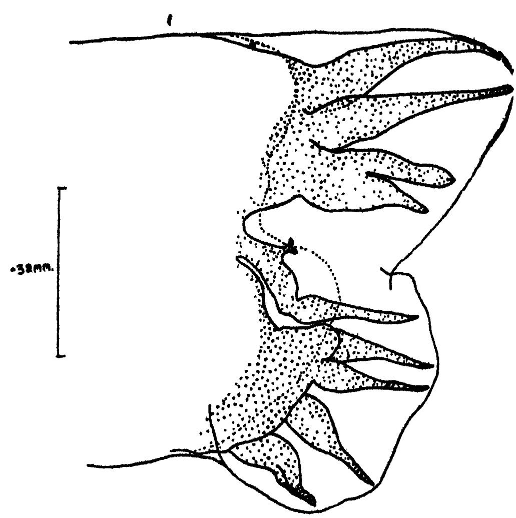
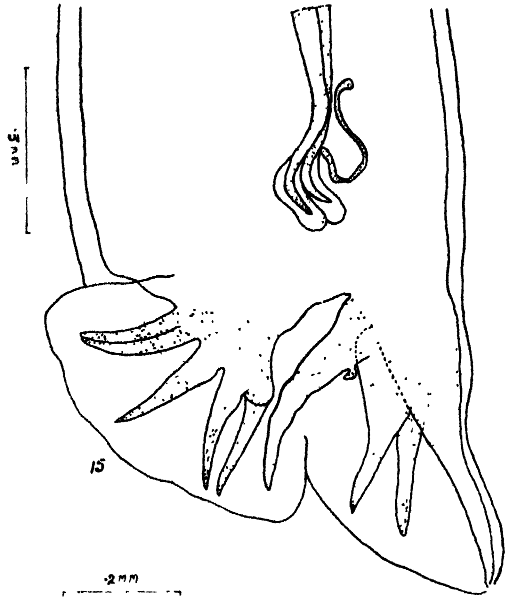


Fig. 14.—*Murshidia falcifera*—male bursa, lateral view. (Orig.)



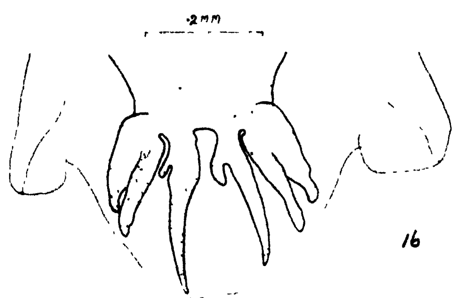
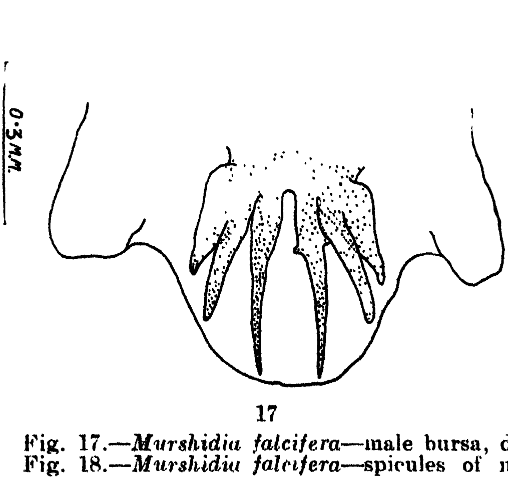


Fig. 15.—*Murshidia falcifera*—male bursa, lateral view. (Orig.)
Fig. 16.—*Murshidia falcifera*—male bursa, dorsal ray variation. (Orig.)



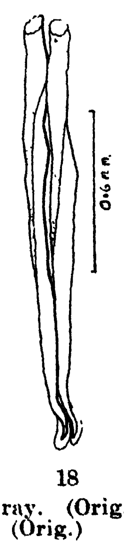


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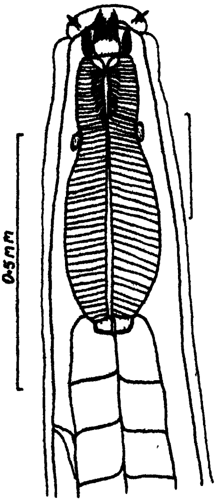
Fig. 17.—*Murshidia falcifera*—male bursa, dorsal ray. (Orig.)
Fig. 18.—*Murshidia falcifera*—spicules of male. (Orig.)



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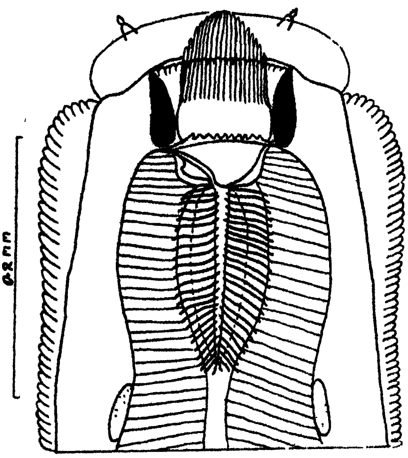

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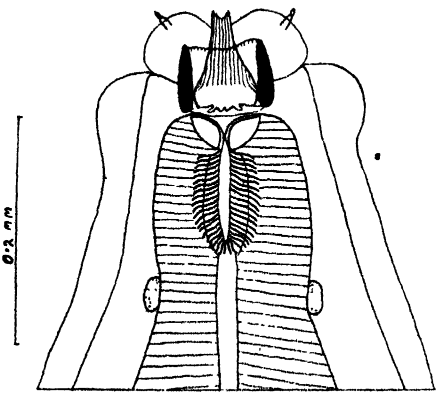
Fig. 19.—*Murshidia indica*—anterior end, dorsal view. (Orig.)
Fig. 20.—*Murshidia indica*—head, lateral view. (Orig.)



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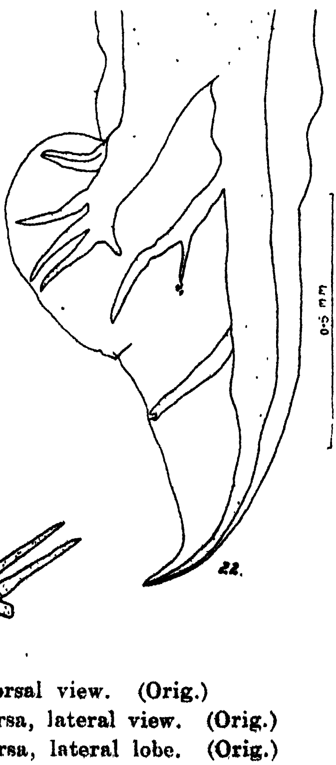

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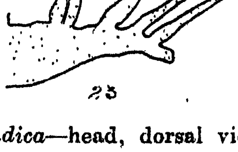

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Fig. 21.—*Murshidia indica*—head, dorsal view. (Orig.)
Fig. 22.—*Murshidia indica*—male bursa, lateral view. (Orig.)
Fig. 23.—*Murshidia indica*—male bursa, lateral lobe. (Orig.)

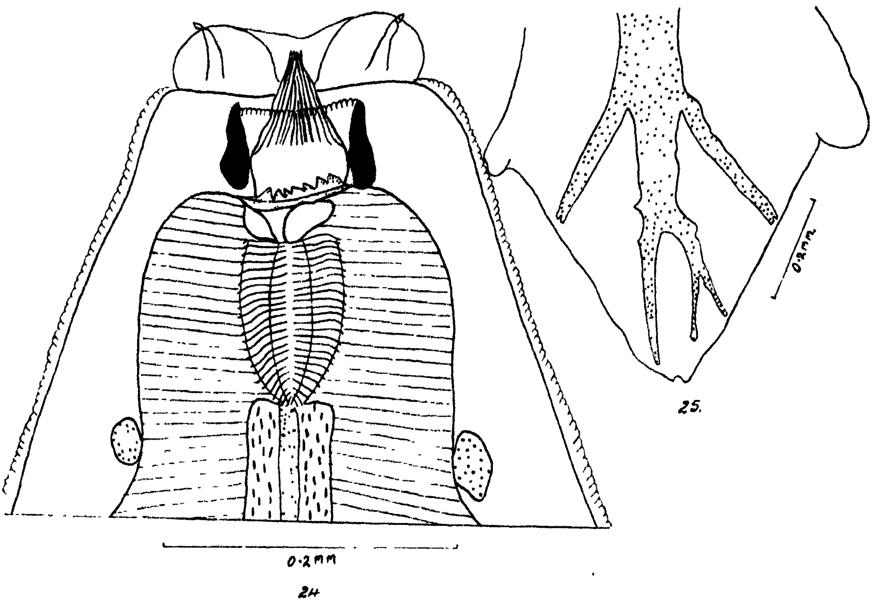


Fig. 24.—*Murshidia indica*—head, dorsal view. (Orig.)
Fig. 25.—*Murshidia indica*—male bursa, dorsal ray. (Orig.)

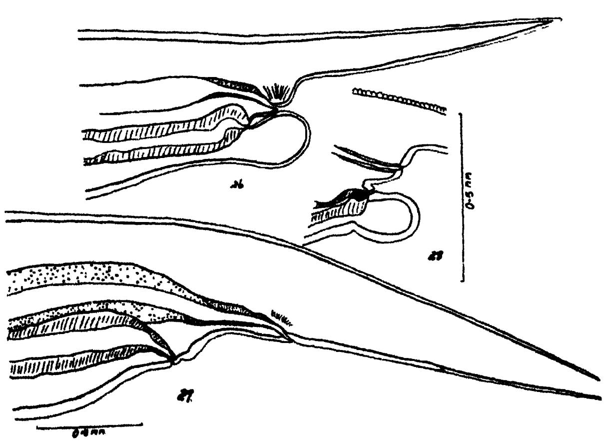
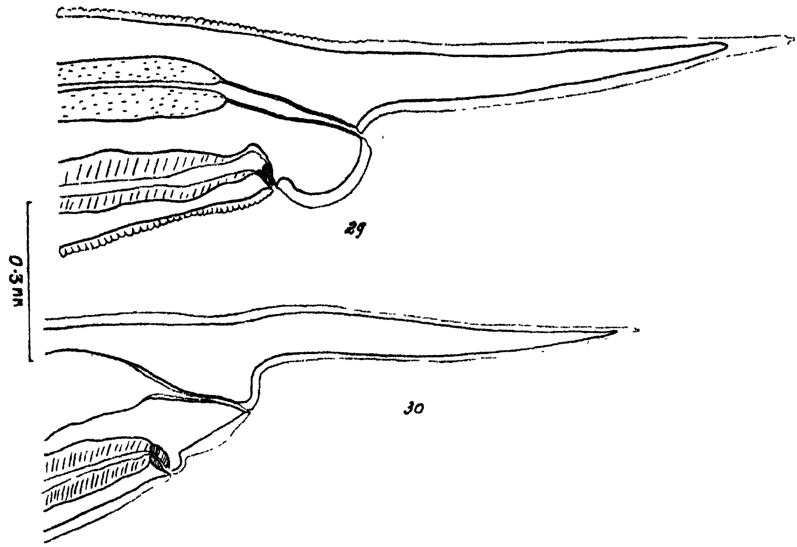
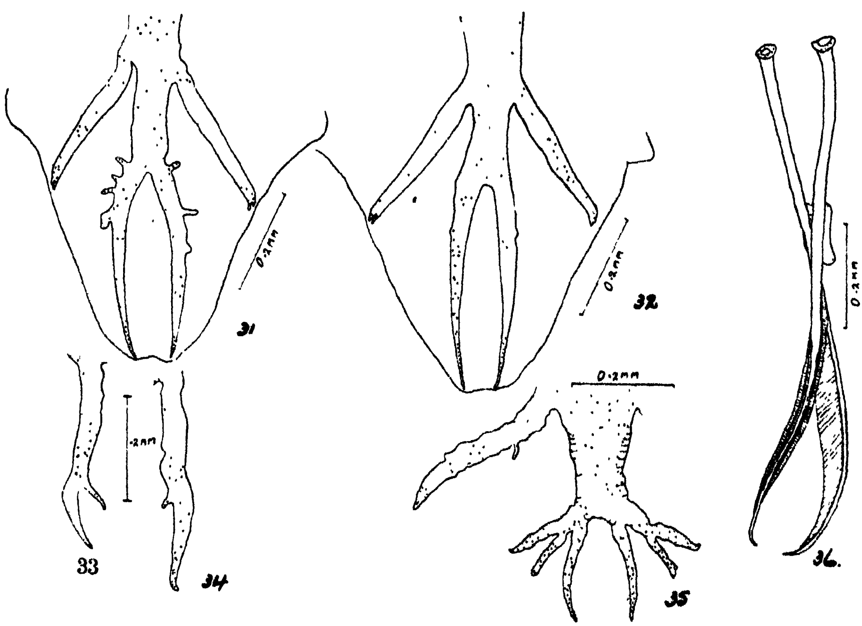


Fig. 26.—*Murshidia indica*—female tail. (Orig.)
Fig. 27.—*Murshidia neveu-lemairei*—female tail. (Orig.)
Fig. 28.—*Murshidia indica*—female, vulvar region. (Orig.)



Figs. 29, 30.—*Murshidia indica*—female tail variations. (Orig.)



Figs. 31, 32.—*Murshidia indica*—male bursa, dorsal ray variations, (Orig.)
Figs. 33, 34.—*Murshidia indica*—male bursa, externo-dorsal ray variations. (Orig.)
Fig. 35.—*Murshidia neveu-lemairei*—male, bursa, dorsal lobe. (Orig.)
Fig. 36:—*Murshidia indica*—spicules of male. (Orig.)

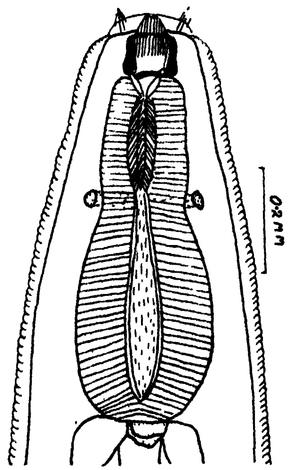


Fig. 37.—*Murshidia neveu-lemairei* anterior end, lateral view. (Orig.)
Fig. 38.—*Murshidia neveu-lemairei*—a sub-median papilla. (Orig.)

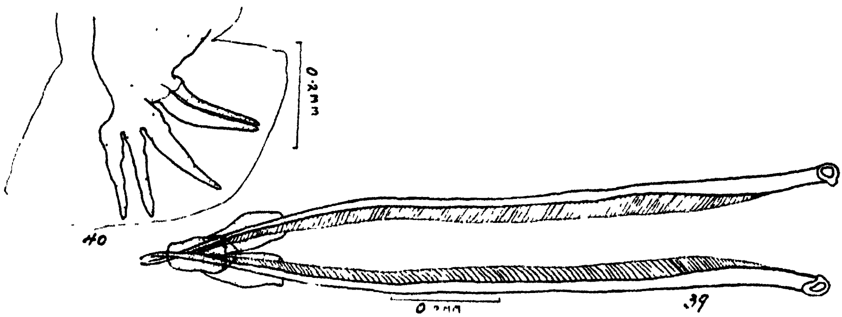
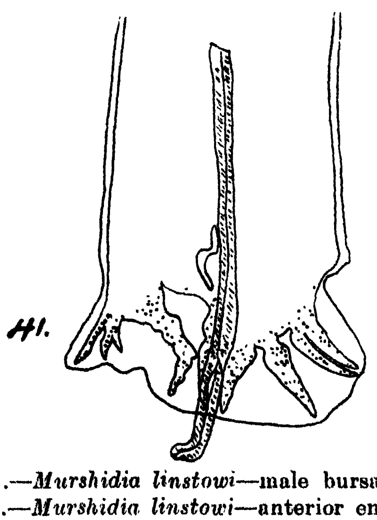


Fig. 39.—*Murshidia neveu-lemairei*—spicules of male. (Orig.)
Fig. 40.—*Murshidia neveu-lemairei*—male bursa, lateral lobe. (Orig.)



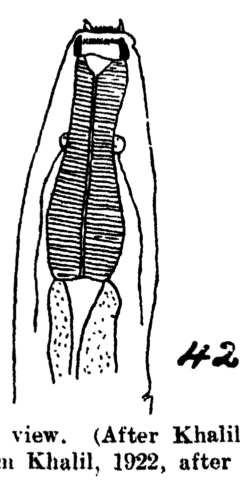
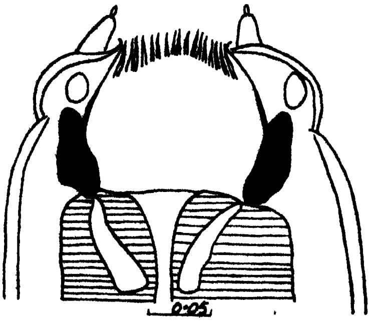


Fig. 41.—*Murshidia linstowi*—male bursa, lateral view. (After Khalil, 1922.)
Fig. 42.—*Murshidia linstowi*—anterior end. (From Khalil, 1922, after Leiper.)



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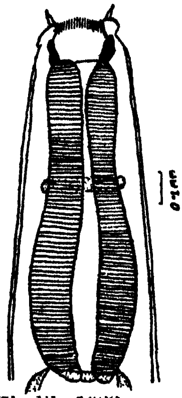

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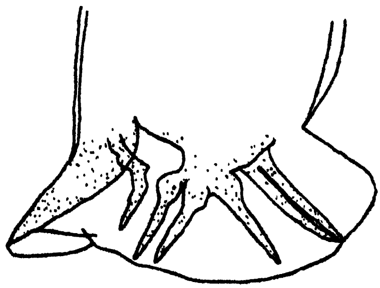
Fig. 43.—*Murshidia hadia*—head. (After Khalil, 1922.)
Fig. 44.—*Murshidia hadia*—anterior end. (After Khalil, 1922.)



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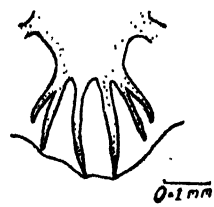

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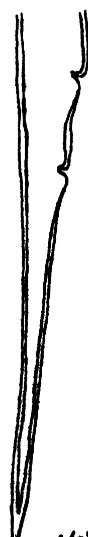

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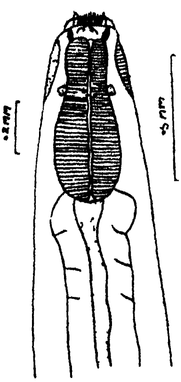
Fig. 45.—*Murshidia hadia*—male bursa, lateral view. (After Khalil, 1922.)
Fig. 46.—*Murshidia hadia*—male bursa, dorsal ray. (After Khalil, 1922.)
Fig. 47.—*Murshidia hadia*—spicules of male. (After Khalil, 1922.)
Fig. 48.—*Murshidia hadia*—female tail. (After Khalil, 1922.)



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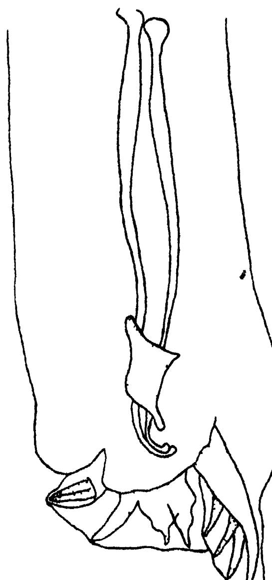

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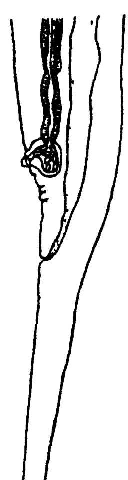
Fig. 49.—*Murshidia longicaudata*—anterior end. (After Neveu-Lemaire, 1928.)
Fig. 50.—*Murshidia longicaudata*—male bursa, lateral view. (After Néveu-Lemaire, 1928.)



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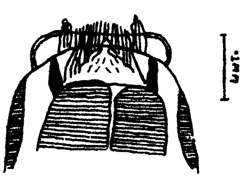

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Fig. 51.—*Murshidia longicaudata*—female tail. (After Neveu-Lemaire, 1928.)
Fig. 52.—*Murshidia longicaudata*—head, lateral view. (After Neveu-Lemaire, 1928.)

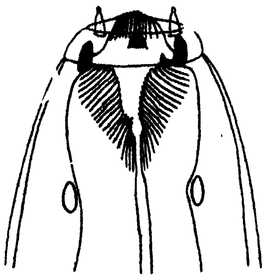
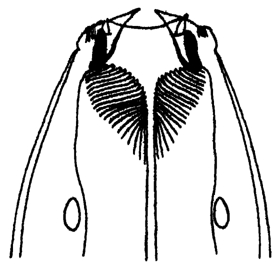

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Fig. 53.—*Murshidia brachyscelis*—anterior end, lateral view. (After Mönnig, 1932.)

Fig. 54.—*Murshidia brachyscelis*—anterior end, ventral view. (After Mönnig, 1932.)


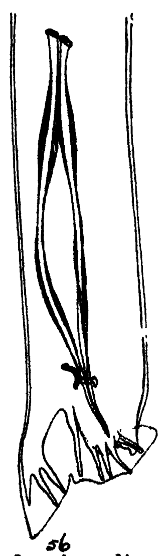
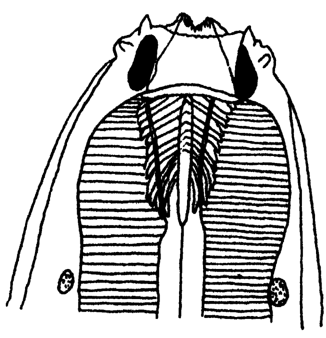
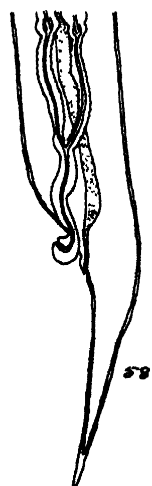

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Fig. 55.—*Murshidia brachyscelis*—male bursa, dorsal view. (Atfer Mönnig, 1932.)

Fig. 56.—*Murshidia brachyscelis*—male bursa, lateral view. (After Mönnig, 1932.)

Fig. 57.—*Murshidia africana*—head, dorsal view. (After Lane, 1921.)

Fig. 58.—*Murshidia africana*—female tail. (After Lane, 1921.)

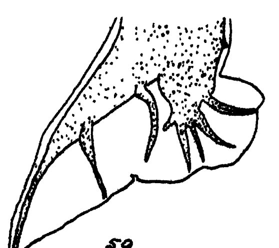
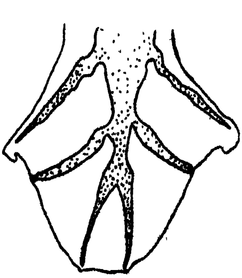

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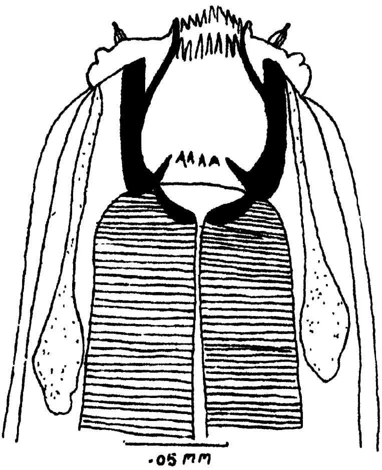
Fig. 59.—*Murshidia africana*—male bursa, lateral view. (After Lane, 1921.)

Fig. 60.—*Murshidia africana*—male bursa, dorsal ray. (After Lane, 1921.)



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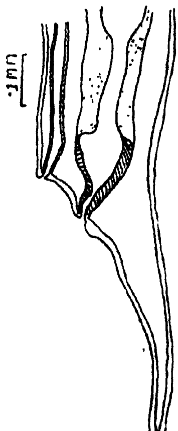

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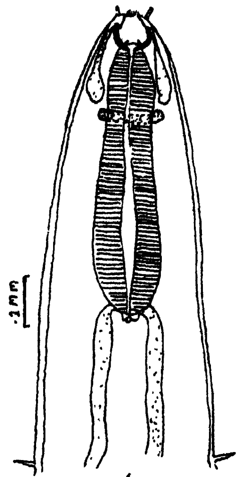

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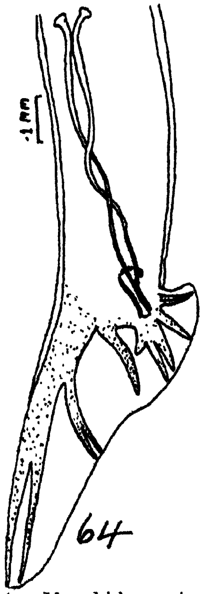
Fig. 61.—*Murshidia anisa*—head, lateral view. (After Khalil, 1922.)
Fig. 62.—*Murshidia anisa*—female tail. (After Khalil, 1922.)
Fig. 63.—*Murshidia anisa*—anterior end. (After Khalil, 1922.



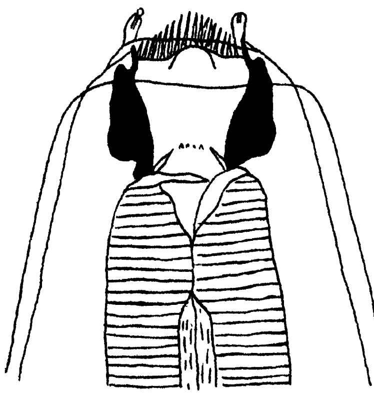


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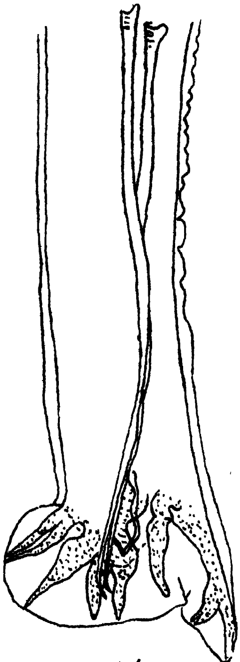
Fig. 64.—*Murshidia anisa*—male bursa, lateral view. (After Khalil, 1922.)
Fig. 65.—*Murshidia dawoodi*—head, lateral view. (After Khalil, 1922.)



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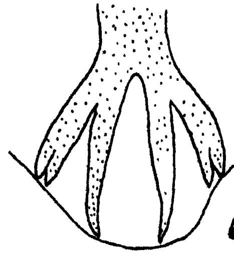

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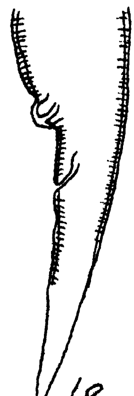

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Fig. 66.—*Murshidia dawoodi*—male bursa, lateral view. (After Khalil, 1922.)
Fig. 67.—*Murshidia dawoodi*—male bursa, dorsal ray. (After Khalil, 1922.)
Fig. 68.—*Murshidia dawoodi*—female tail. (After Khalil, 1922.)

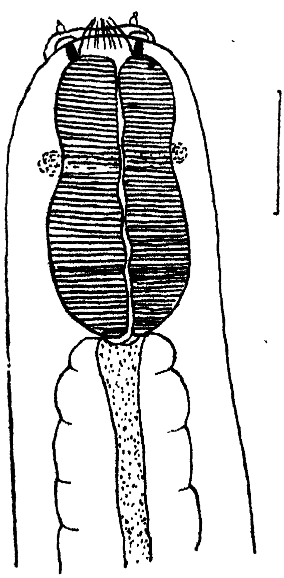

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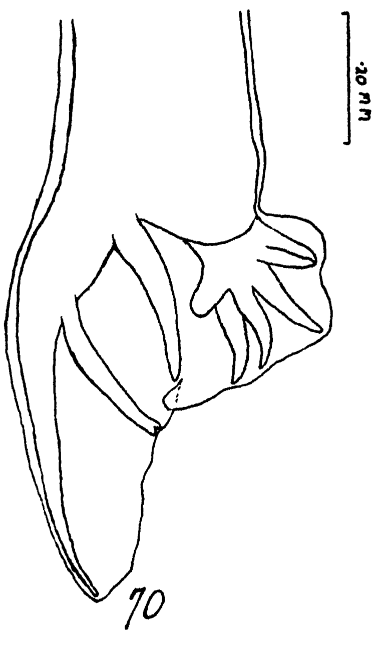
Fig. 69.—*Murshidia omoensis*—anterior end, lateral view. (After Neveu-Lemaire, 1928.)



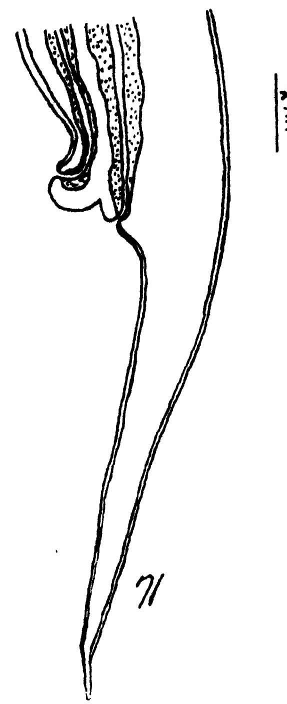


Fig. 70.—*Murshidia omoensis*—male bursa, lateral view. (After Neveu-Lemaire, 1928.)

Fig. 71.—*Murshidia omoensis*—female tail. (After Neveu-Lemaire, 1928.)

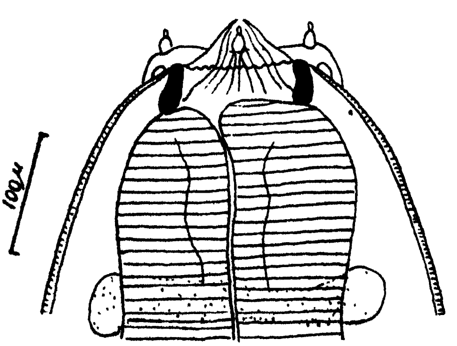


Fig. 72.—*Murshidia omoensis*—(From African Rhinoceros.)—head, dorsal view. (After Neveu-Lemaire, 1924.)

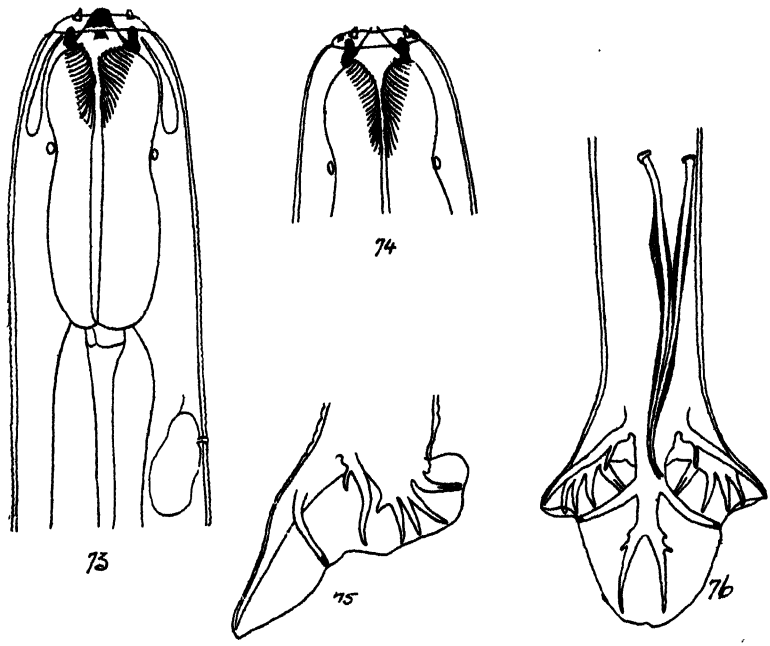
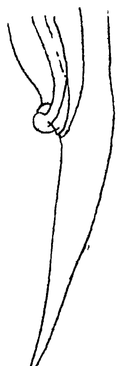


Fig. 73.—*Murshidia brevicapsulatus*—anterior end, lateral view. (After Mönnig, 1932.)

Fig. 74.—*Murshidia brevicapsulatus*—head, dorsal view. (After Mönnig, 1932.)

Fig. 75.—*Murshidia brevicapsulatus*—male bursa, lateral view. (After Mönnig, 1932.)

Fig. 76.—*Murshidia brevicapsulatus*—male bursa, dorsal view. (After Mönnig, 1932.)



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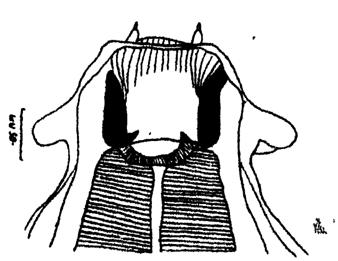

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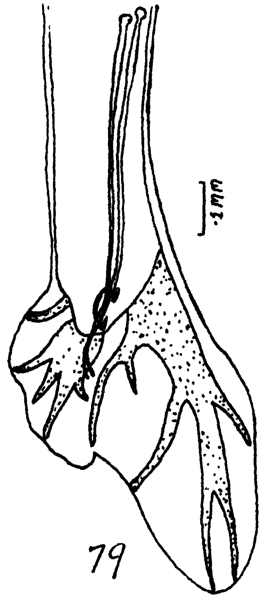
Fig. 77.—*Murshidia brevicapsulutus*—female tail. (After Mönnig, 1932.)

Fig. 78.—*Murshidia memphisia*—head, lateral view. (After Khalil, 1922.)



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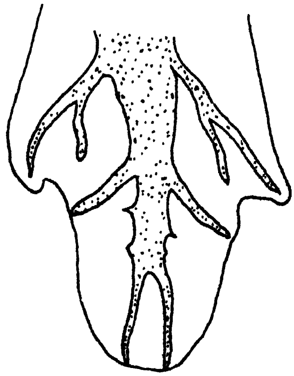

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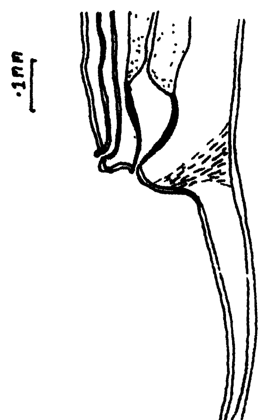
Fig. 79.—*Murshidia memphisia*—male bursa, lateral view. (After Khalil, 1922.)
Fig. 80.—*Murshidia memphisia*—male bursa, dorsal ray. (After Khalil, 1922.)



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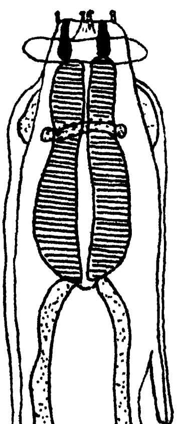

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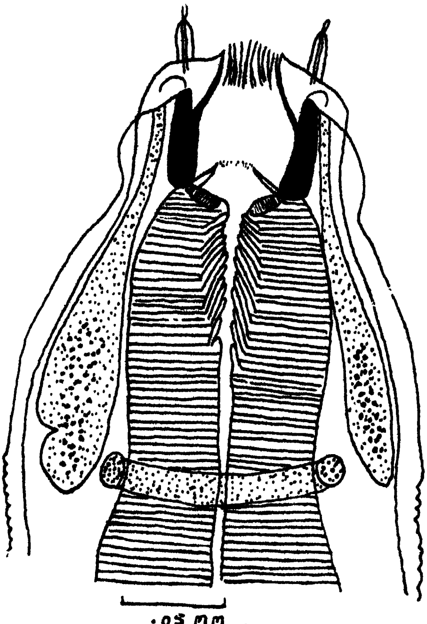
Fig. 81.—*Murshidia memphisia*—female tail. (After Khalil, 1922.)
Fig. 82.—*Murshidia memphisia*—anterior end, lateral view. (After Khalil, 1922.)



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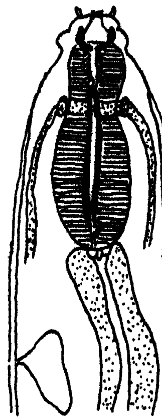

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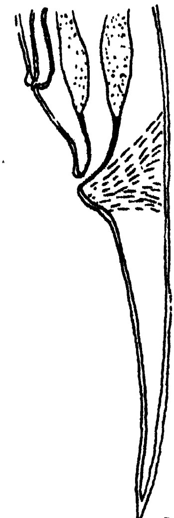

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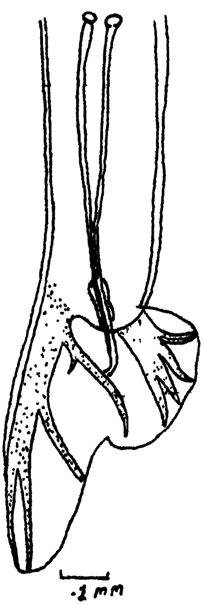
Fig. 83.—*Murshidia aziza*—head, lateral view. (After Khalil, 1922.)
Fig. 84.—*Murshidia aziza*—anterior end, lateral view. (After Kalil, 1922.)
Fig. 85.—*Murshidia aziza*—female tail. (After Khalil, 1922.)



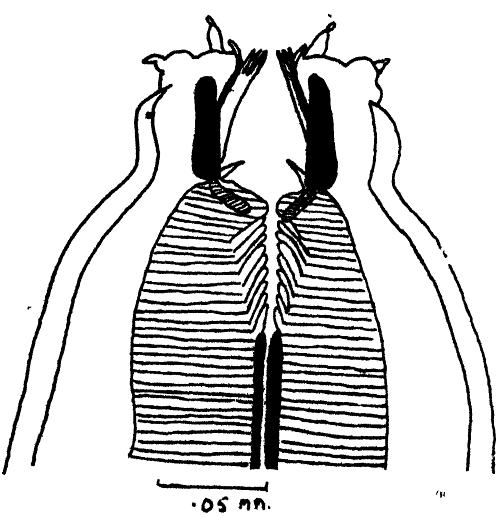


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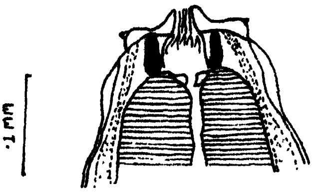
Fig. 86.—*Murshidia aziza*—male bursa, lateral view. (After Khalil, 1922.)
Fig. 87.—*Murshidia aziza*—head, dorsal view. (After Khalil, 1922.)



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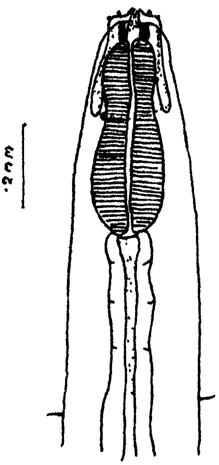

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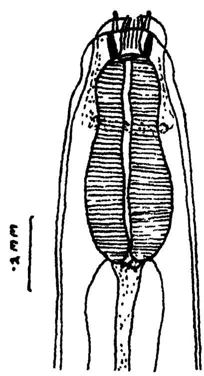

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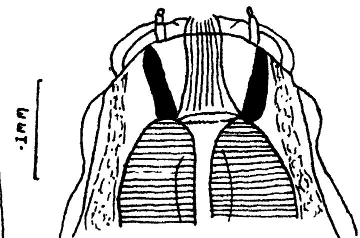

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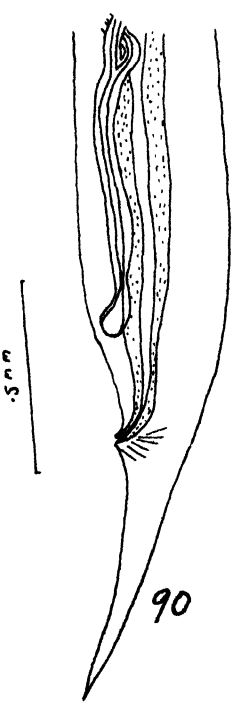

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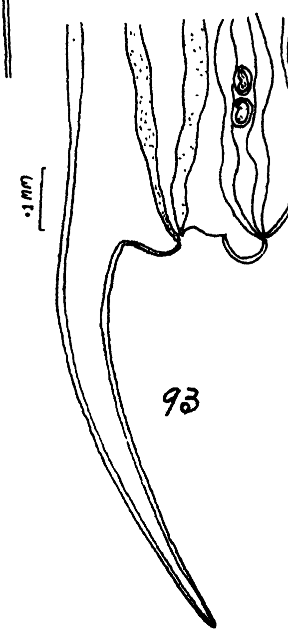

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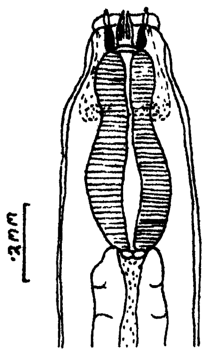
Fig. 88.—*Murshidia loxodontae*—head, dorsal view. (After Neveu-Lemaire, 1928.)
Fig. 89.—*Murshidia loxodontae*—anterior end, dorsal view. (After Neveu-Lemaire, 1928.)
Fig. 90.—*Murshidia loxodontae*—female tail. (After Neveu-Lemaire, 1928.)
Fig. 91.—*Murshidia soudanensis*—anterior end. (After Neveu-Lemaire, 1928.)
Fig. 92.—*Murshidia soudanensis*—head, lateral view. (After Neveu-Lemaire, 1928.)
Fig. 93.—*Murshidia soudanensis*—female tail. (After Neveu-Lemaire, 1928.)



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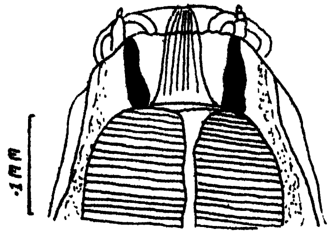

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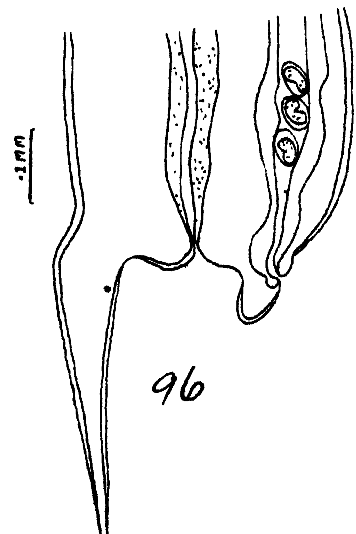


Fig. 94.—*Murshidia brevicauda*—anterior end. (After Neveu-Lemaire, 1928.)

Fig. 95.—*Murshidia brevicauda*—head, dorsal view. (After Neveu-Lemaire, 1928.)

Fig. 96.—*Murshidia brevicauda*—female tail. (After Neveu-Lemaire, 1928.)

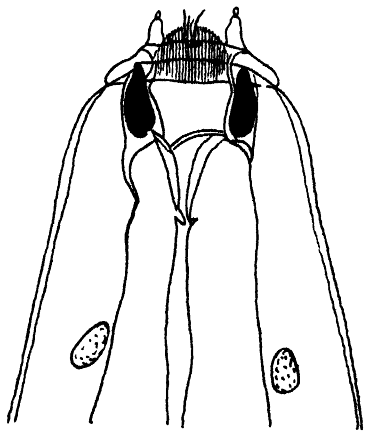
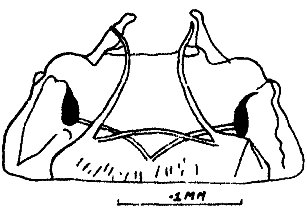


Fig. 97.—*Murshidia lanei*—head, lateral view. (After Witenberg, 1925.)



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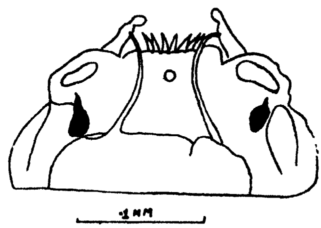

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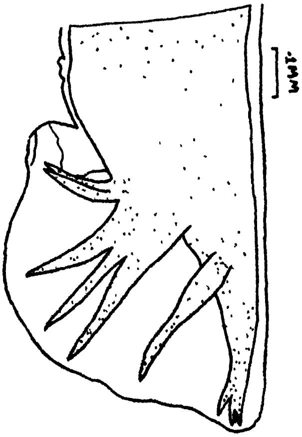
Fig. 98.—*Quilonia renniei*—head, dorsal view. (After Lane, 1914.)
Fig. 99.—*Quilonia renniei*—head, lateral view. (After Lane, 1914.)



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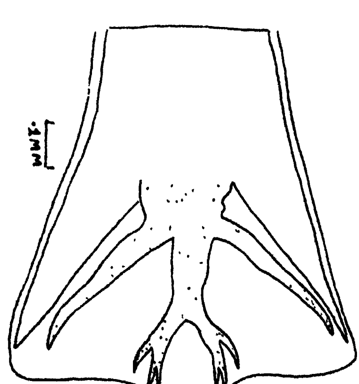

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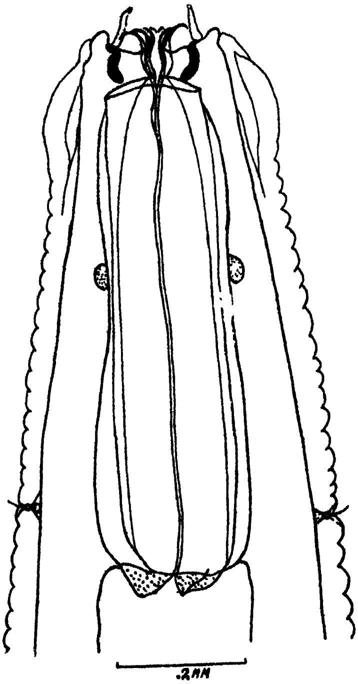
Fig. 100.—*Quilonia renniei*—male bursa, lateral view. (After Lane, 1914.)
Fig. 101.—*Quilonia renniei*—male bursa, dorsal ray. (After Lane, 1914.)



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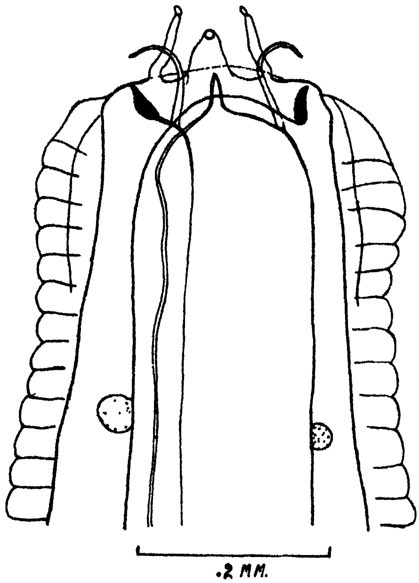

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Fig. 102.—*Quilonia travancra*—anterior end, dorsal view. (Orig.)
Fig. 103.—*Quilonia travancra*—head, lateral view. (Orig.)

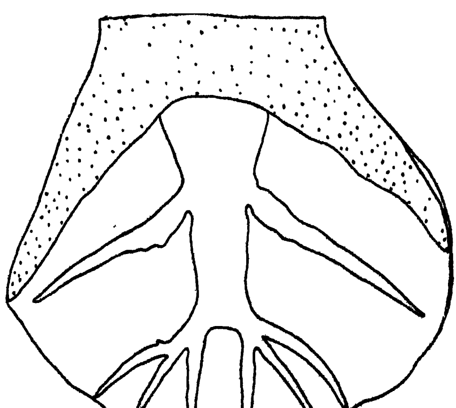
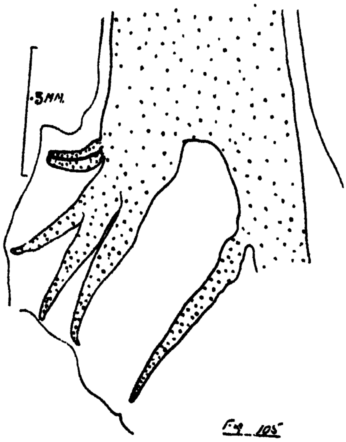


Fig. 104.—*Quilonia travancra*—male bursa, dorsal ray, ventral view. (Orig.)



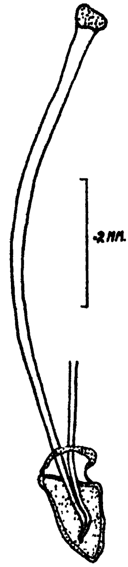


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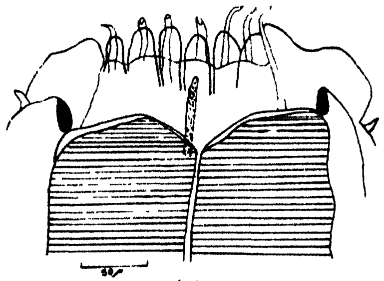
Fig. 105.—*Quilonia travancra*—male bursa, lateral ray. (Orig.)
Fig. 106.—*Quilonia travancra*—spicules of male bursa. (Orig.)



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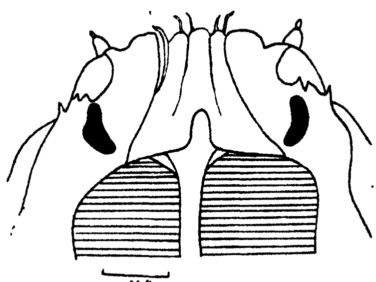

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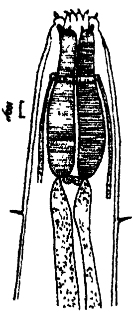

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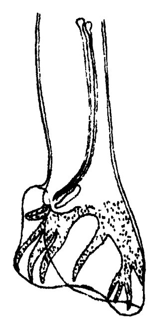

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Fig. 107.—*Quilonia apiensis*—head, lateral view. (After Khalil, 1922.)
Fig. 108.—*Quilonia apiensis*—head, dorsal view. (After Khalil, 1922.)
Fig. 109.—*Quilonia apiensis*—anterior end, ventral view. (After Khalil, 1922.)
Fig. 110.—*Quilonia apiensis*—female tail. (After Khalil, 1922.)
Fig. 111.—*Quilonia apiensis*—male bursa, lateral view. After Khalil, 1922.)
Fig. 112.—*Quilonia apiensis*—male bursa, dorsal ray. (After Khalil, 1922.)

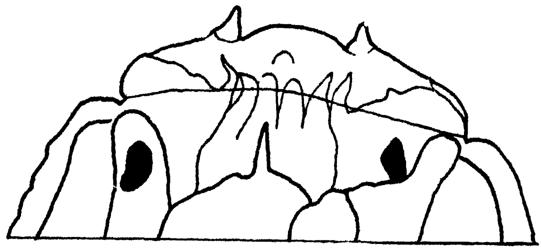

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Fig. 113.—*Quilonia africana*—head, lateral view. (After Lane, 1921.)

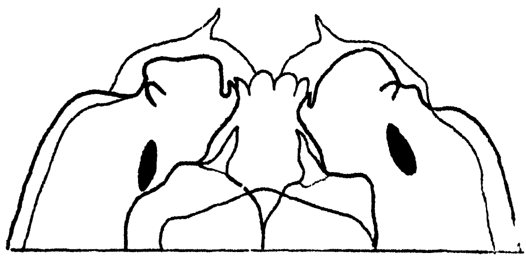

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Fig. 114.—*Quilonia africana*—head, ventral view. (After Lane, 1921.)

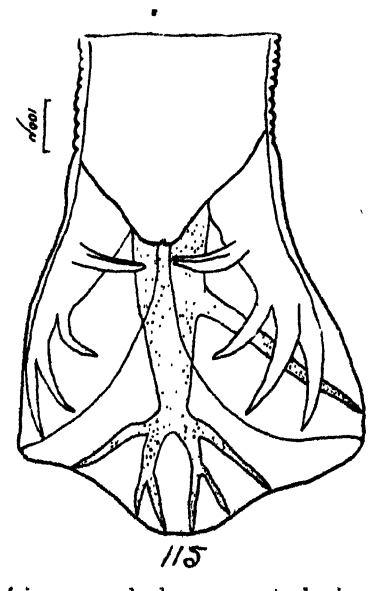

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Fig. 115.—*Quilonia africana*—male bursa, ventral view. (After Khalil, 1922.)

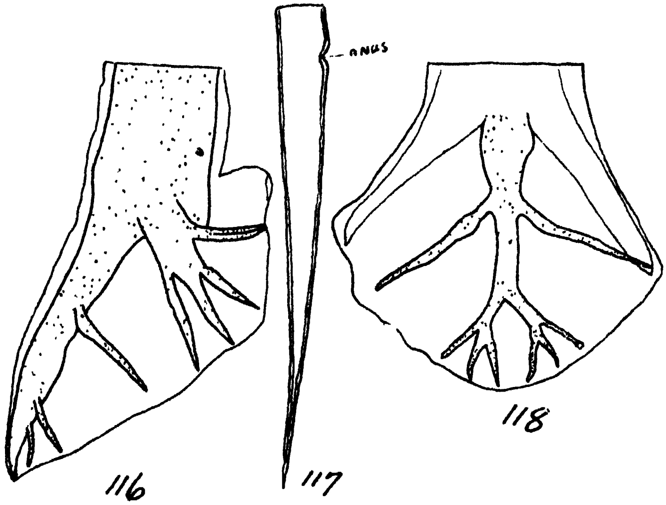

Fig. 116.—*Quilonia africana*—male bursa, lateral view. (After Lane, 1921.)
Fig. 117.—*Quilonia africana*—female tail. (After Lane, 1921.)
Fig. 118.—*Quilonia africana*- male bursa, dorsal ray, dorsal view. (After Lane, 1921.)

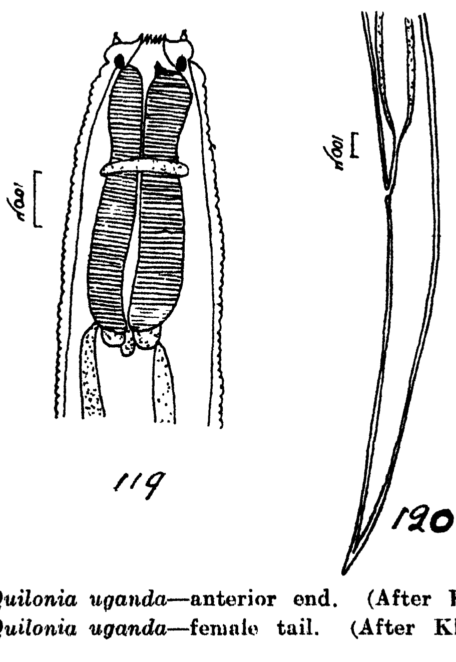

Fig. 119.—*Quilonia uganda*—anterior end. (After Khalil, 1922.)
Fig. 120.—*Quilonia uganda*—female tail. (After Khalil, 1922.)

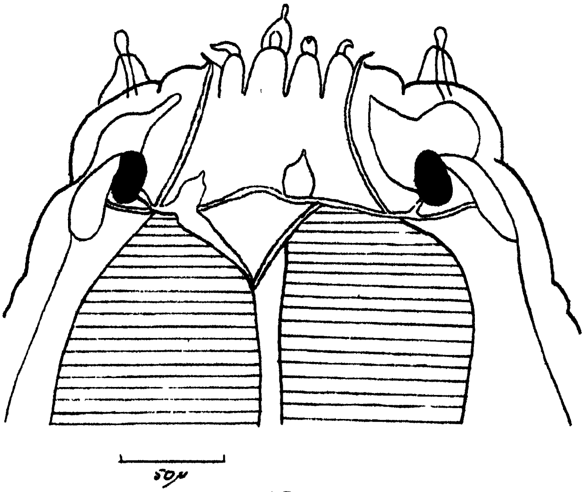

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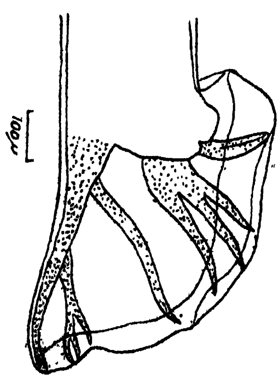
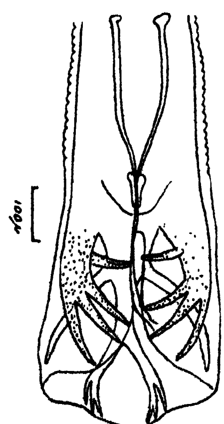
Fig. 121.—*Quilonia uganda*—head. (After Khalil, 1922.)





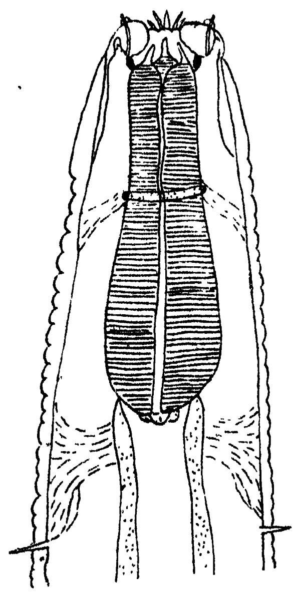


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Fig. 122.—*Quilonia uganda*—male bursa, lateral view. (After Khalil, 1922.)
Fig. 123.—*Quilonia uganda*—male bursa, ventral view. (After Khalil, 1922.)
Fig. 124.—*Quilonia brevicauda*—anterior end, dorsal view. (After Khalil, 1922.)

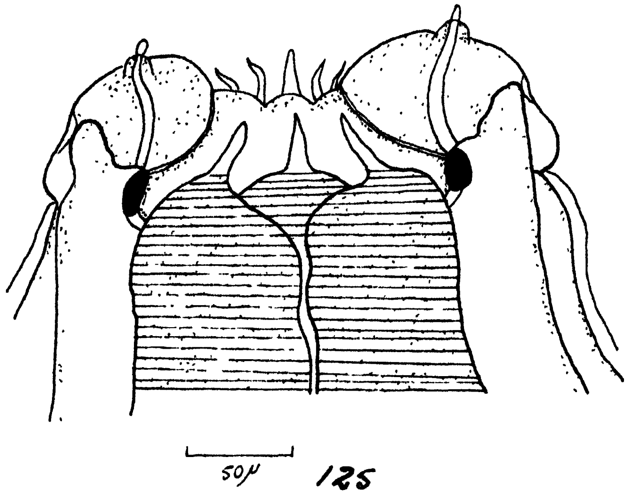
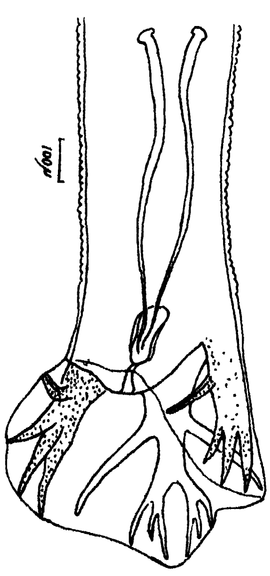


Fig. 125.—*Quilonia brevicauda*—head. (After Khalil, 1922.)



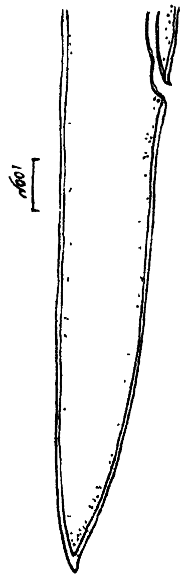


Fig. 126.—*Quilonia brevicauda*—male bursa, ventral view. (After Khalil, 1922.)
Fig. 127.—*Quilonia brevicauda*—female tail. (After Khalil, 1922.)

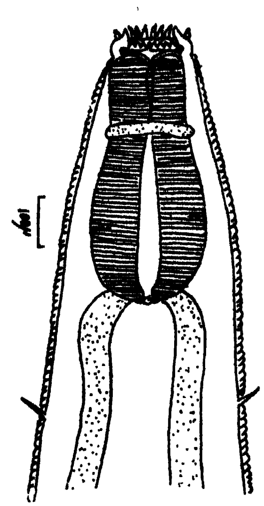


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Fig. 128.—*Quilonia ethiopica*—anterior end, dorsal view. (After Khalil, 1922.)
Fig. 129.—*Quilonia ethiopica*—female tail. (After Khalil, 1922.)

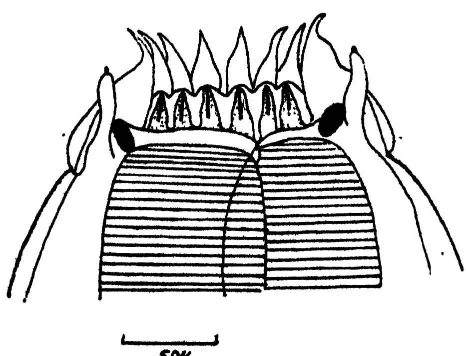

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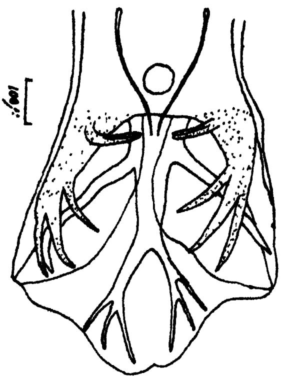
Fig. 130.—*Quilonia ethiopica*—head. (After Khalil, 1922.)



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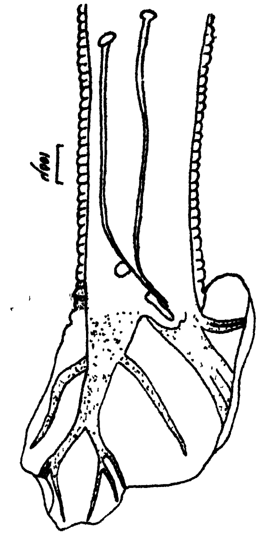

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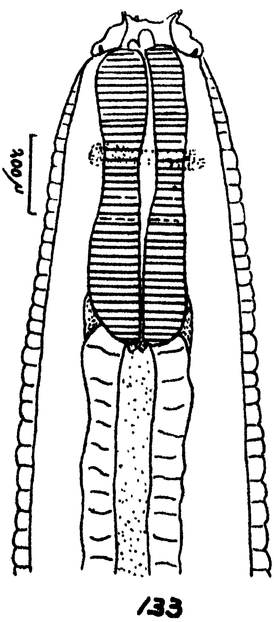
Fig. 131.—*Quilonia ethiopica*—male bursa, ventral view. (After Khalil, 1922.)
Fig. 132.—*Quilonia ethiopica*—male bursa, lateral view. (After Khalil, 1922.)



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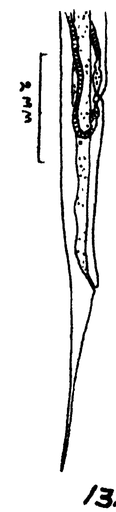

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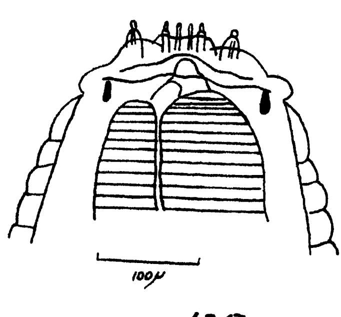

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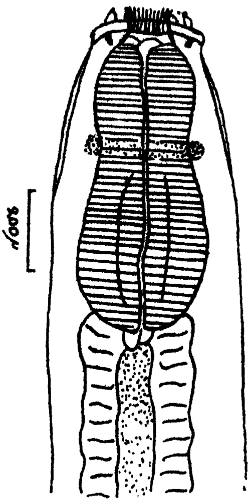
Fig. 133.—*Quilonia khalili*—anterior end. (After Neveu-Lemaire, 1928.)
Fig. 134.—*Quilonia khalili*—female tail. (After Neveu-Lemaire, 1928.)
Fig. 135.—*Quilonia khalili*—head. (After Neveu-Lemaire, 1928.)



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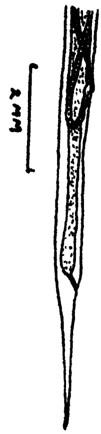

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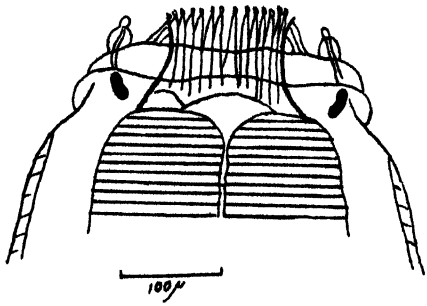

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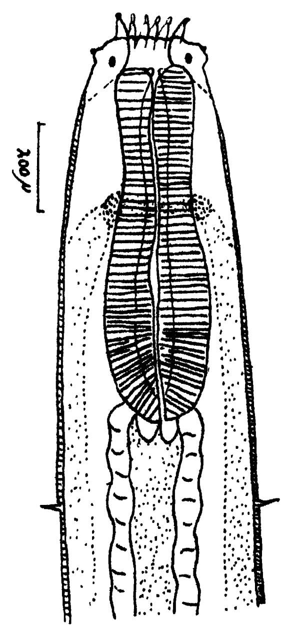
Fig. 136.—*Quilonia loxodontae*—anterior end. (After Neveu-Lemaire, 1928.)
Fig. 137.—*Quilonia loxodontae*—female tail. (After Neveu-Lemaire, 1928.)
Fig. 138.—*Quilonia loxodontae*—head. (After Neveu-Lemaire, 1928.)



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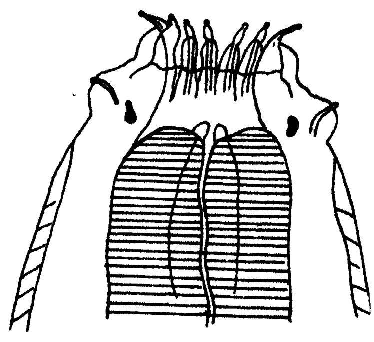

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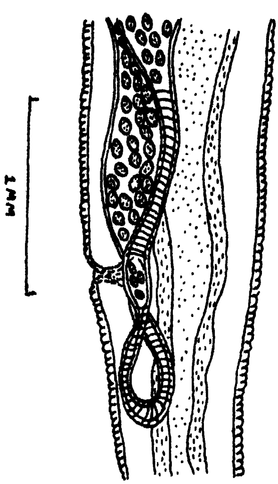
Fig. 139.—*Quilonia magna*—anterior end, ventral view. (After Neveu-Lemaire, 1928.)
Fig. 140.—*Quilonia magna*—head. (After Neveu-Lemaire, 1928.)



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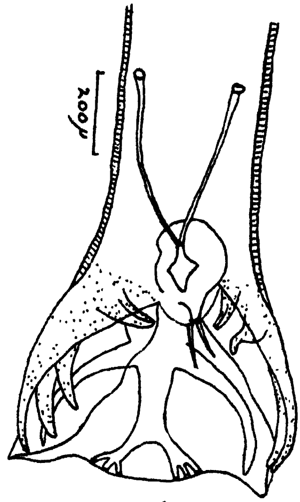

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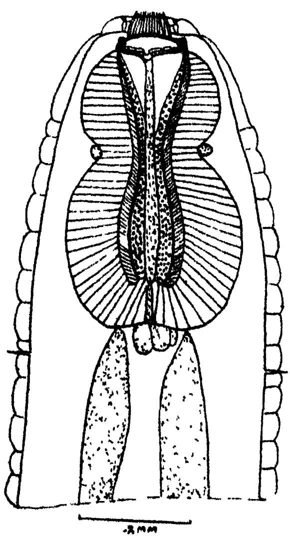
Fig. 141.—*Quilonia magna*—female uteri. (After Neveu-Lemaire, 1928.)

Fig. 142.—*Quilonia magna*—male bursa, ventral view. (After Neveu-Lemaire, 1928.)

Fig. 143.—*Quilonia magna*—male bursa, dorsal ray digitation. (After Neveu-Lemaire, 1928.)



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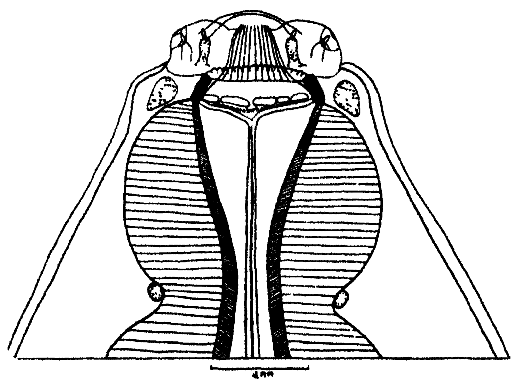

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Fig. 144.—*Amira pileata*—anterior end, dorsal view. (Orig.)

Fig. 145.—*Amira pileata*—head, dorsal view. (Orig.)

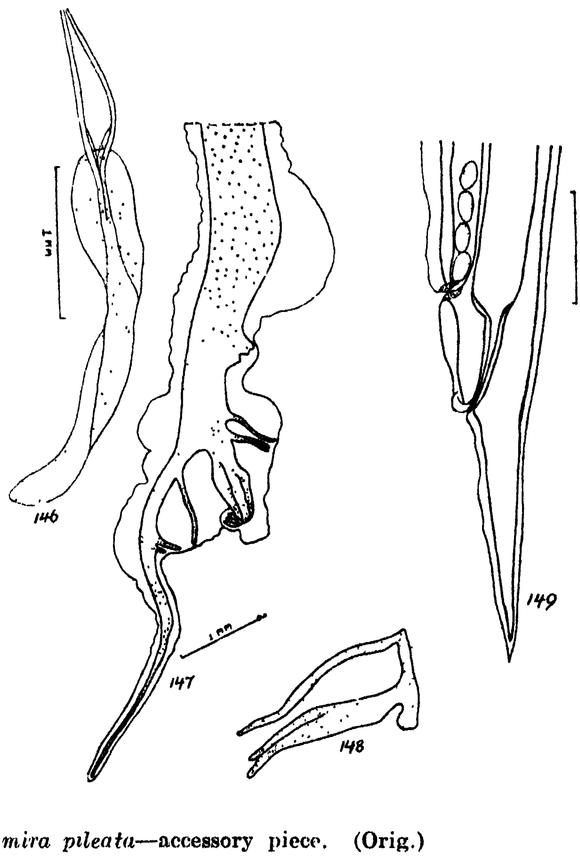
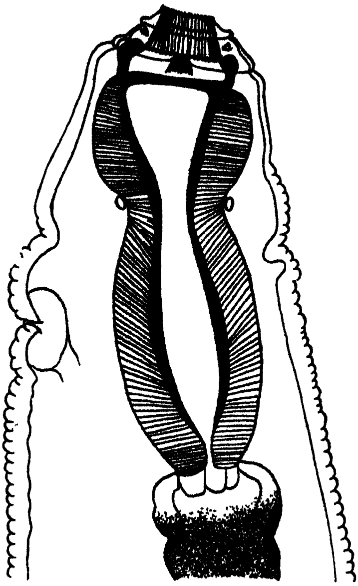


Fig. 146.—*Amira pileata*—accessory piece. (Orig.)

Fig. 147.—*Amira pileata*—male bursa, lateral view. (After Khalil, 1922, and Lane, 1914.)

Fig. 148.—*Amira pileata*—male bursa, ventral ray. (After Lane, 1914.)

Fig. 149.—*Amira pileata*—female tail. (Orig.)



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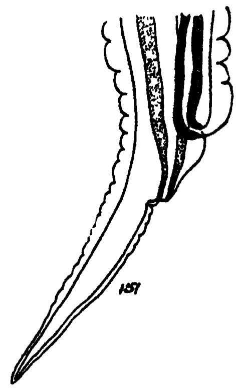
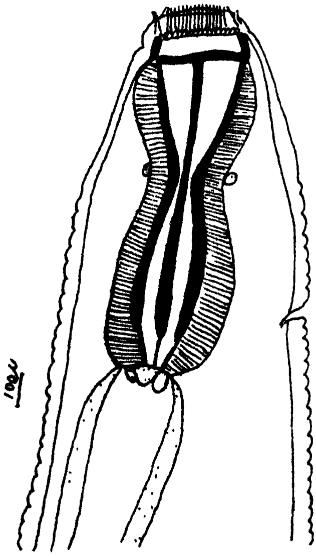


Fig. 150.—*Amira sameera*—anterior end, lateral view. (After Mönnig, 1932.)
Fig. 151.—*Amira sameera*—female tail. (After Mönnig, 1932.)



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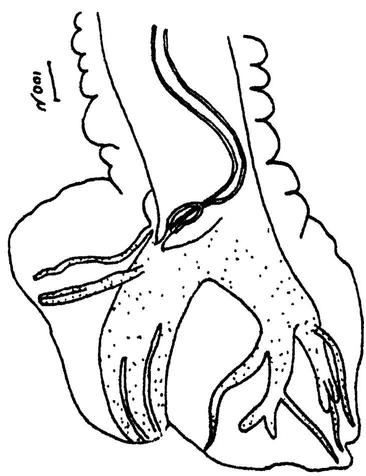

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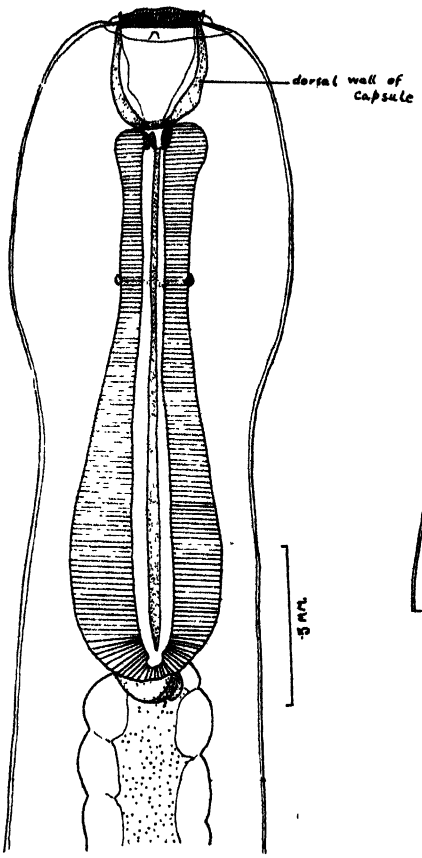
Fig. 152.—*Amira sameera*—anterior end, lateral view. (After Khalil, 1922.)
Fig. 153.—*Amira sameera*—male bursa, lateral view. (After Khalil, 1922.)



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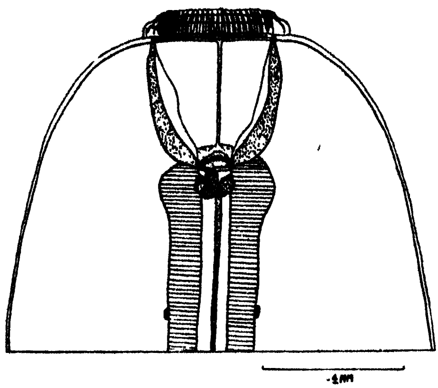

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Fig. 154.—*Equinubria sipunculiformis*—anterior end, lateral view. (Orig.)
Fig. 155.—*Equinubria sipunculiformis*—head, dorsal view. (Orig.)

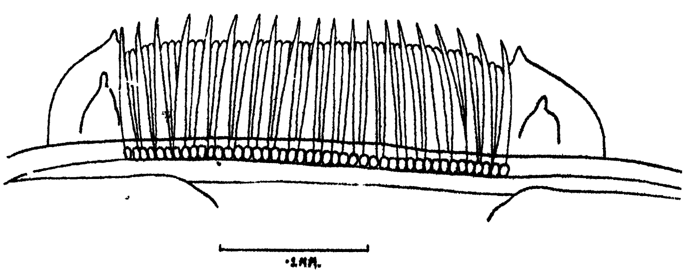


Fig. 156.—*Equinubria sipunculiformis*—anterior extremity. (Orig.)

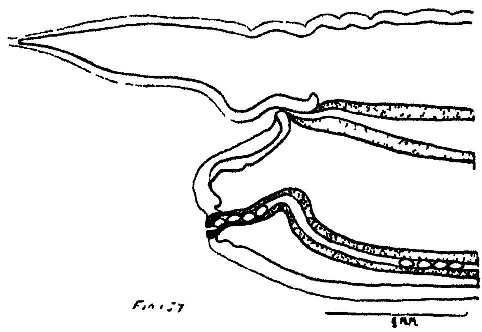
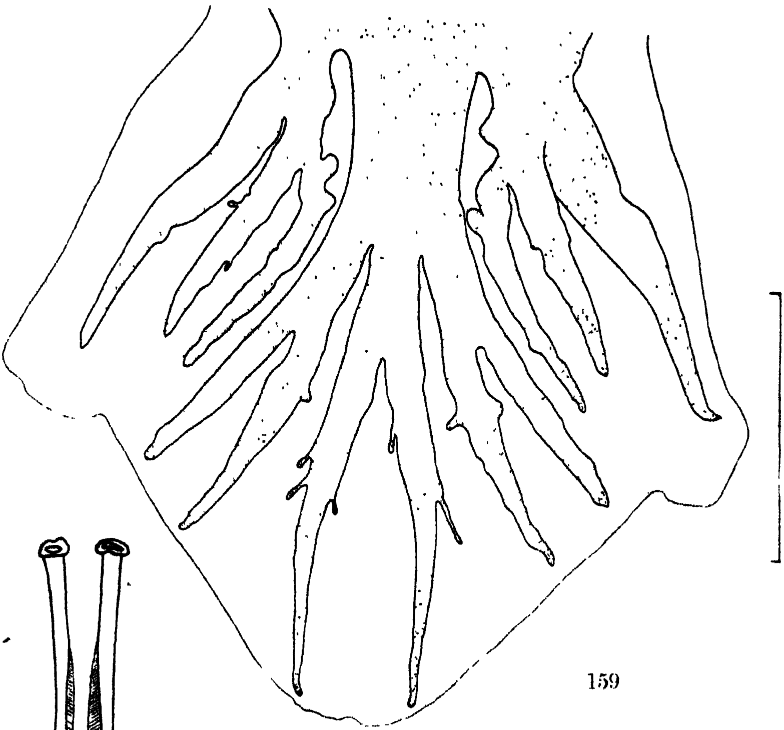


Fig. 157.—*Equinubria sipunculiformis*—female tail. (Orig.)

Fig. 158.—*Equinubria sipunculiformis*—male bursa, lateral view. (Orig.)



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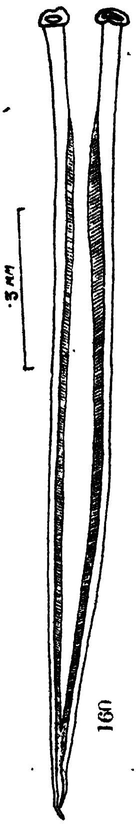

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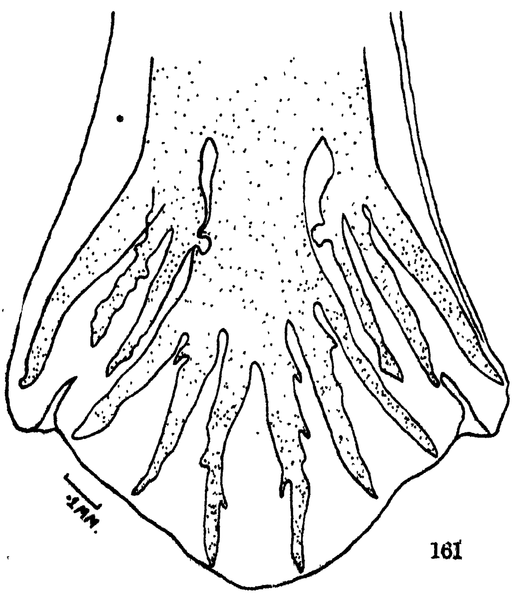

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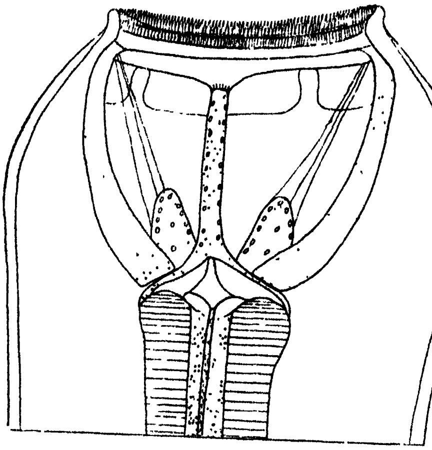
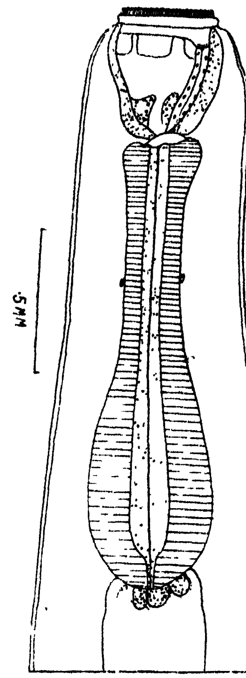
Fig. 159.—*Equinubria sipunculiformis*—male bursa, dorsal lobe. (Orig.)
Fig. 160.—*Equinubria sipunculiformis*—spicules of male. (Orig.)
Fig. 161.—*Equinubria sipunculiformis*—male bursa, dorsal lobe. (After Lane, 1914.)





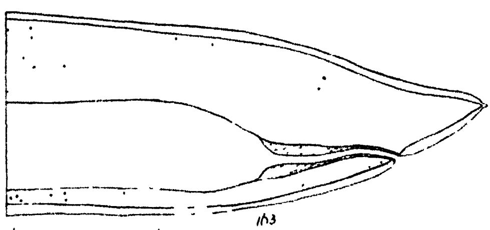
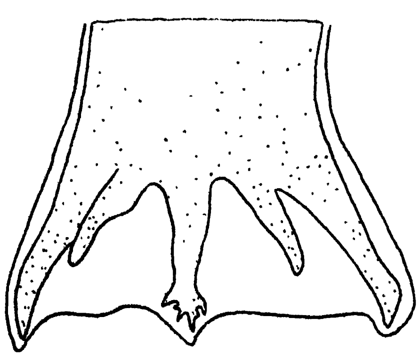


Fig. 162.- *Decrusia additictia*—head, dorsal view. (Orig.)
Fig. 163.—*Decrusia additictia*—female tail. (Orig.)
Fig. 164.—*Decrusia additictia*—anterior end, lateral view. (Orig.)



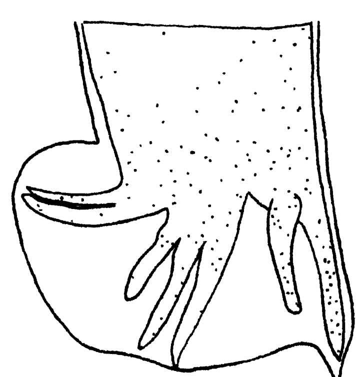


Fig. 165.—*Decrusia additictia*—male bursa, dorsal lobe. (After Lane, 1914.)
Fig. 166.—*Decrusia addictia*—male bursa, lateral view. (After Lane, 1914.)

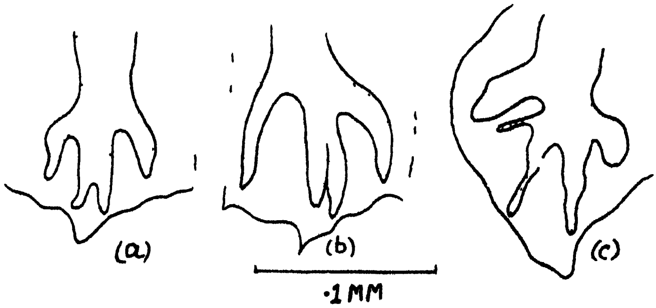
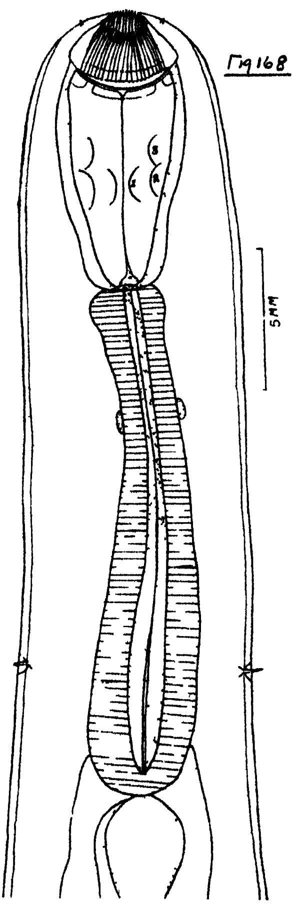


Fig. 167 (*a*) (*b*) (*c*).—*Decrusia additicta*—variations of dorsal ray tip. (After Lane, 1914.)



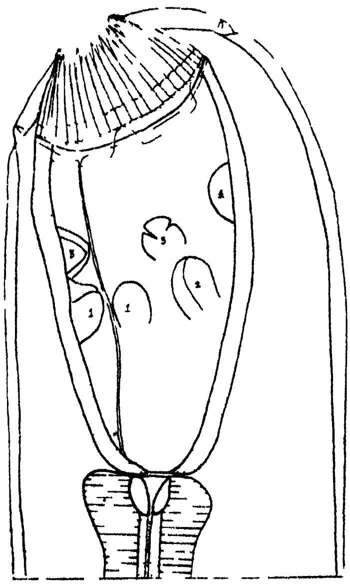


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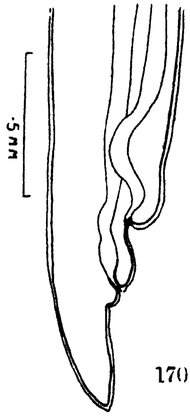
Fig. 168.—*Choniangium epistomum*—anterior end, dorsal view. (Orig.)
Fig. 169.—*Choniangium epistomum*—head, dorso-lateral view. (Orig.)



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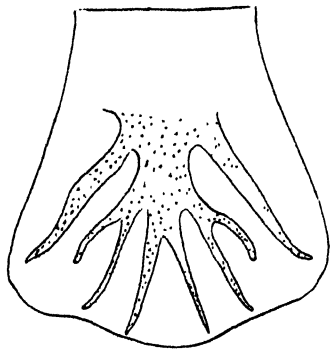

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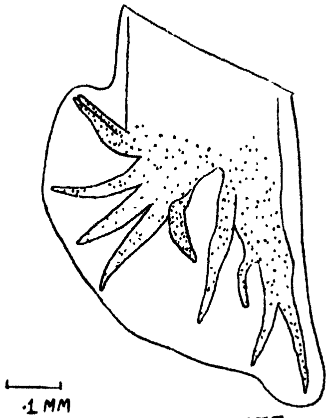

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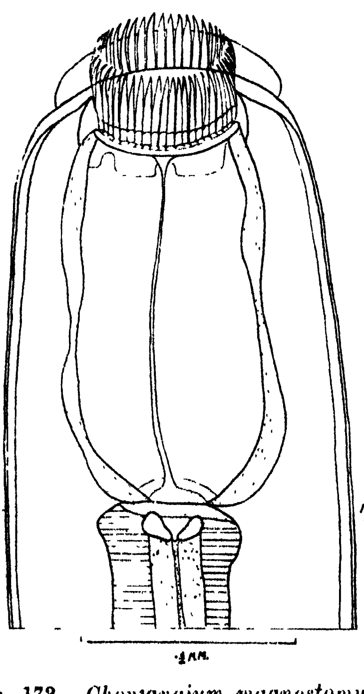
Fig. 170.—*Choniangium epistomum*—female tail. (Orig.)

Fig. 171.—*Choniangium epistomum*—male bursa, dorsal ray. (After Lane, 1914.)

Fig. 172.—*Choniangium epistomum*—male bursa, lateral view. (After Lane, 1914.)



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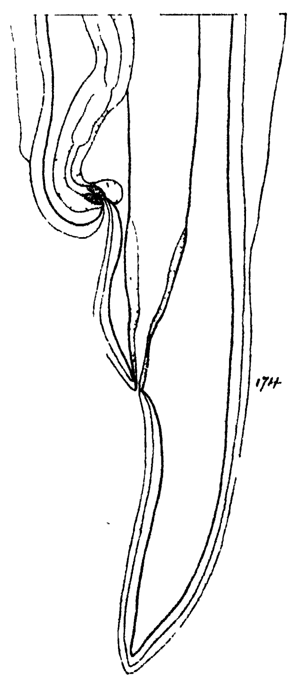

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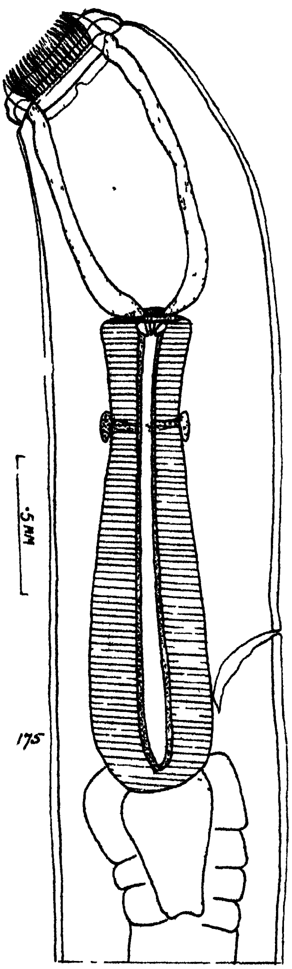
Fig. 173.—*Choniangium magnostomum*—n.sp.—anterior end, dorsal view. (Orig.)

Fig. 174.—*Choniangium magnostomum*—n.sp.—female tail. (Orig.)



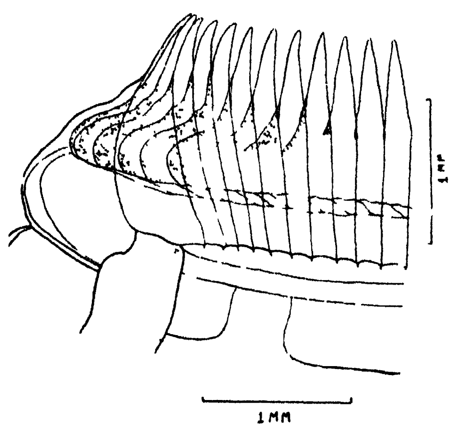


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Fig. 175.—*Choniangium magnostomum*—n.sp.—anterior end, lateral view. (Orig.)

Fig. 176.—*Choniangium magnostomum*—n.sp.—portion of leafcrown. (Orig.)

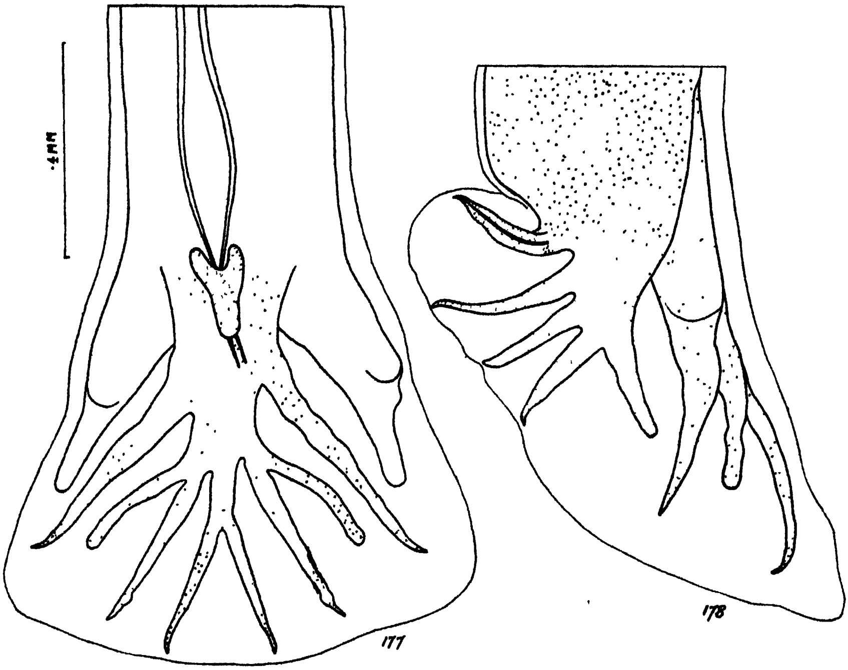


Fig. 177.—*Choniangium magnostomum*—n.sp.—male bursa, dorsal view. (Orig.)
Fig. 178.—*Choniangium magnostomum*—n.sp. —male bursa, lateral view. (Orig.)

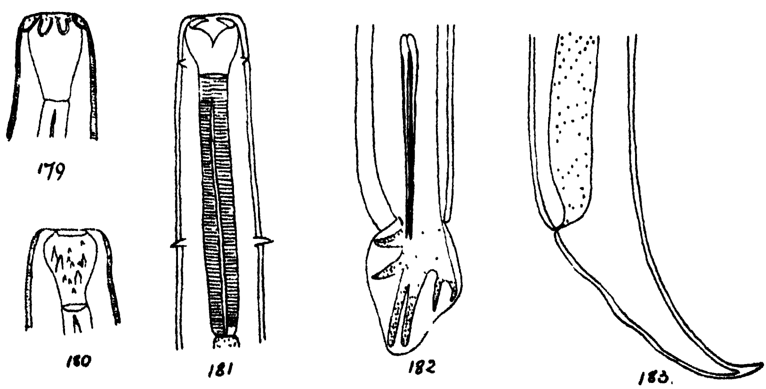


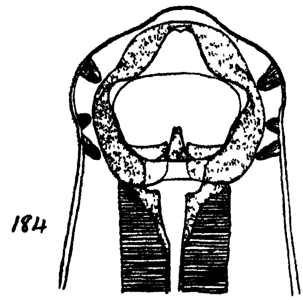
Fig. 179.—*Bunostomum foliatum*—head, showing lateral auricular folds. (After Cobbold, 1882.)

Fig. 180.—*Bunostomum foliatum*—head, lateral view, showing buccal teeth. (After Cobbold, 1882.)

Fig. 181.—*Bunostomum foliatum*—anterior end, ventral view. (After Cobbold, 1882.)

Fig. 182.—*Bunostomum foliatum*—male bursa, lateral view. (After Cobbold, 1882.)

Fig. 183.—*Bunostomum foliatum*—female tail. (After Cobbold, 1882.)



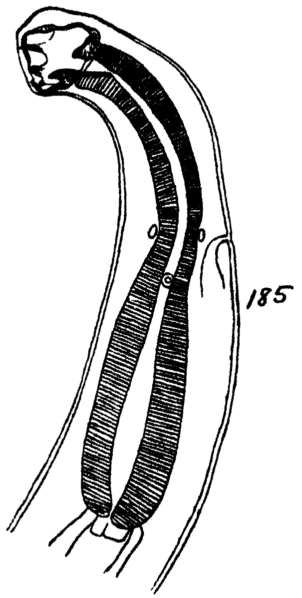
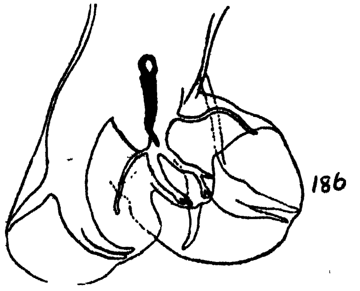


Fig. 184.—*Bunostomum brevispiculum*—head, dorsal view. (After Mönnig, 1932.)

Fig. 185.—*Bunostomum brevispiculum*—anterior end, lateral view. (After Mönnig, 1932.)



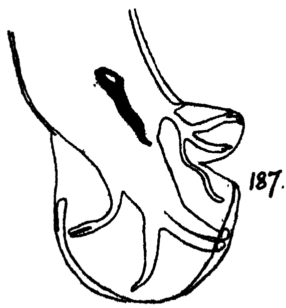
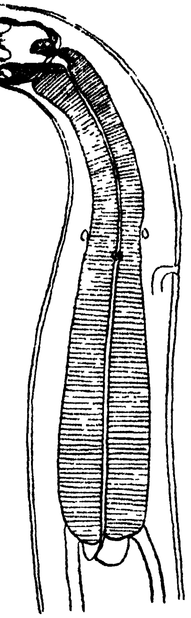


Fig. 186.—*Bunostomum brevispiculum*—male bursa, dorsal view. (After Mönnig, 1932.)

Fig. 187.—*Bunostomum brevispiculum*—male bursa, lateral view. (Atfer Mönnig, 1932.)



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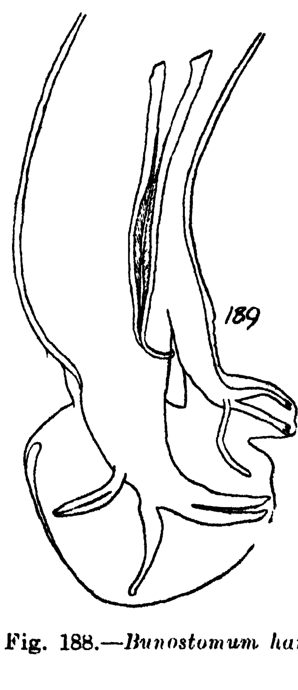

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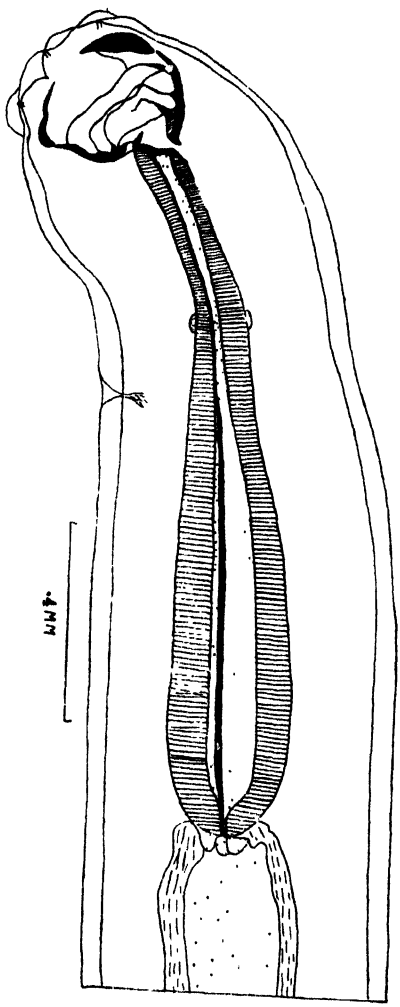

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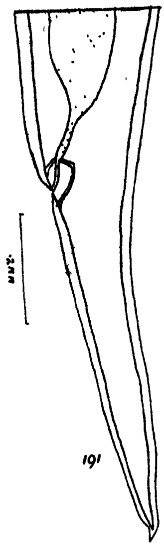
Fig. 188.—*Bunostomum hamatum*—anterior end, lateral view. (After Mönnig, 1932.)

Fig. 189.—*Bunostomum hamatum*—hind end of male, lateral view. (After Mönnig, 1932.)

Fig. 190.—*Bathmostomum sangeri*—anterior end, lateral view. (Orig.)



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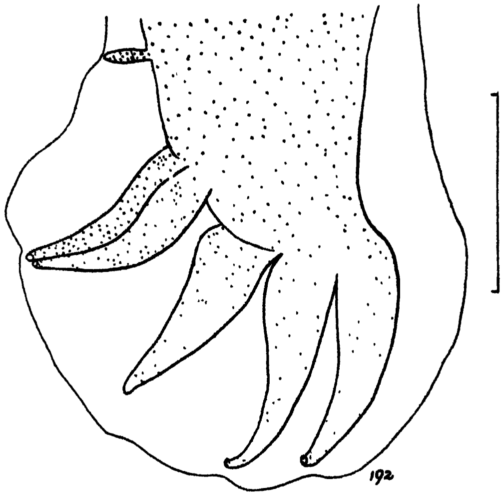

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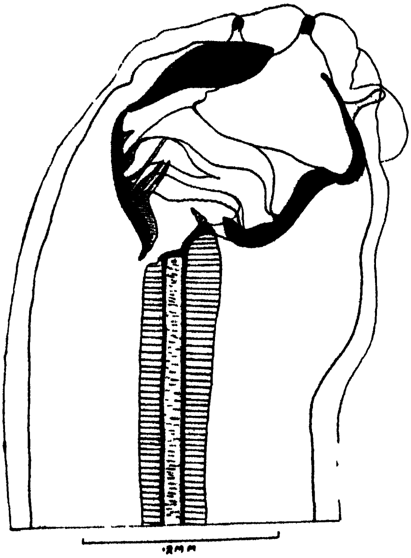

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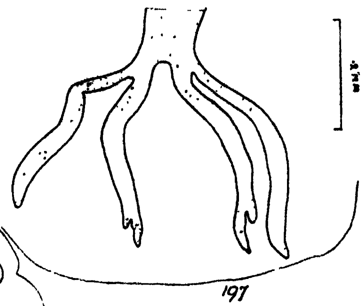
Fig. 191.—*Bathmostomum sangeri*—female tail. (Orig.)
Fig. 192.—*Bathmostomum sangeri*—male bursa, lateral lobe. (Orig.)
Fig. 193.—*Bathmostomum sangeri*—head, lateral view. (Orig.)
Fig. 194.—*Bathmostomum sangeri*—spicules of male. (Orig.)
Fig. 195.—*Bathmostomum sangeri*—spicules of male. (Orig.)



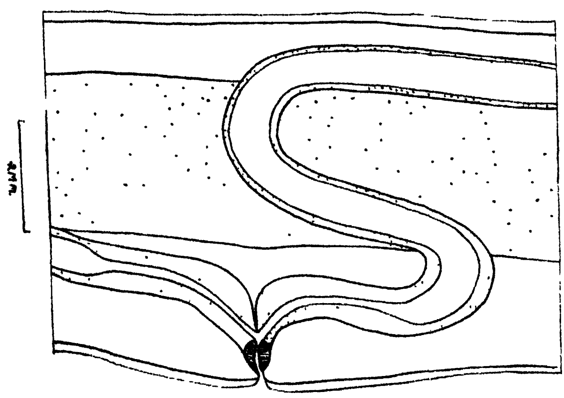


Fig. 196.—*Bathmostomum sangeri*--male bursa, lateral view. (Orig.)

Fig. 197.—*Bathmostomum sangeri*—male bursa, dorsal ray. (Orig.)

Fig. 198.—*Bathmostomum sangeri*—vulvar region. (Orig.)

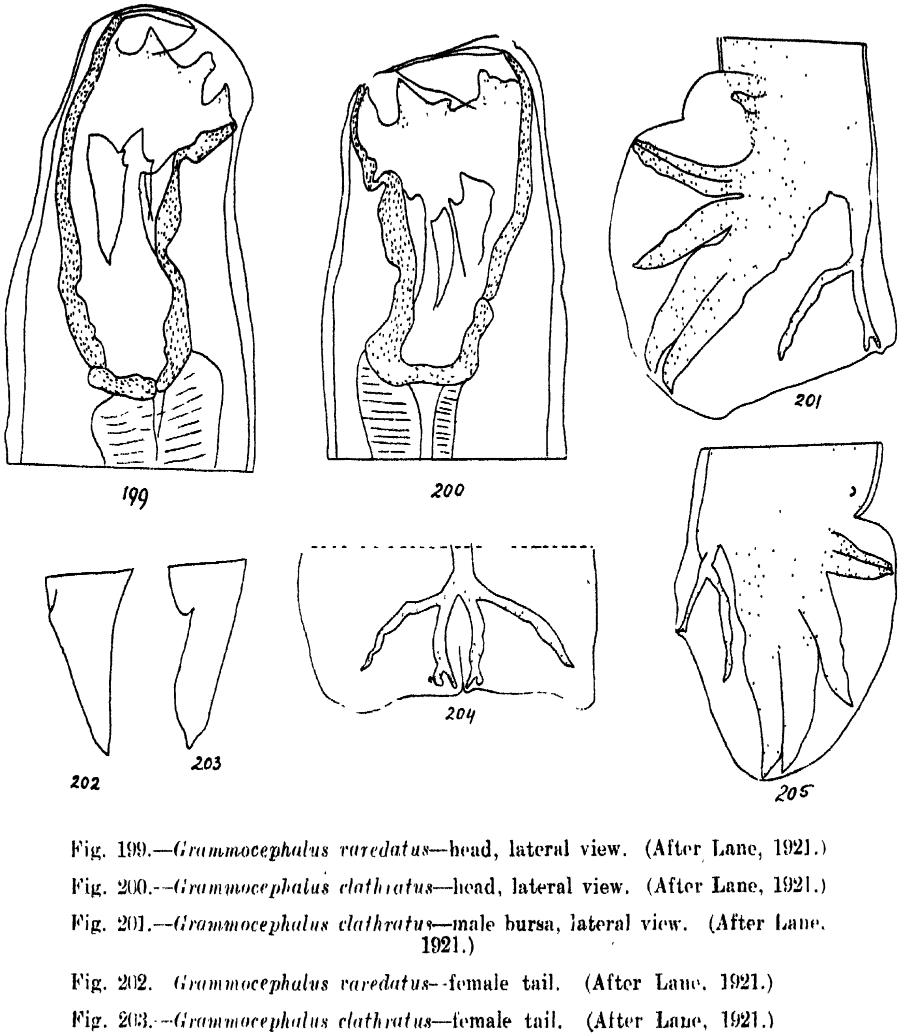


Fig. 199.—*Grammocephalus varedatus*—head, lateral view. (After Lane, 1921.)

Fig. 200.—*Grammocephalus clathratus*—head, lateral view. (After Lane, 1921.)

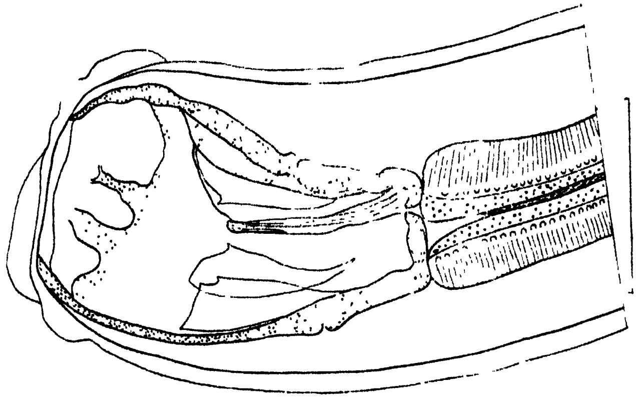
Fig. 201.—*Grammocephalus clathratus*—male bursa, lateral view. (After Lane, 1921.)

Fig. 202. *Grammocephalus varedatus*—female tail. (After Lane, 1921.)

Fig. 203.—*Grammocephalus clathratus*—female tail. (After Lane, 1921.)

Fig. 204.—*Grammocephalus clathratus*—dorsal ray of bursa. (After Lane, 1921.)

Fig. 205.—*Grammocephalus varedatus*—male bursa, lateral view. (After Lane, 1921.)



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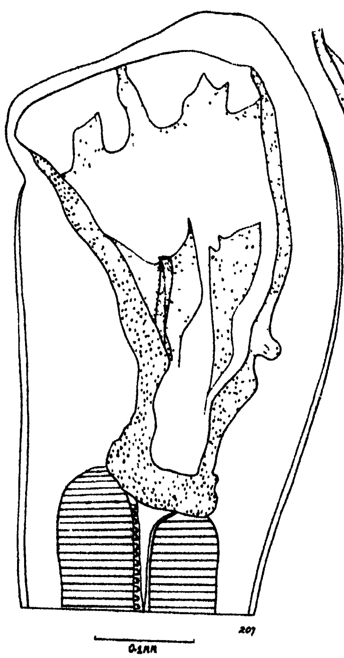


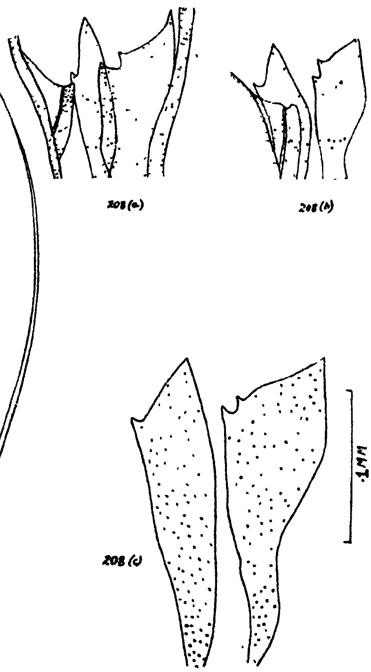


Fig. 206.—*Grammocephalus hybridatus*—n.sp.—anterior end, lateral view. (Orig.)

Fig. 207.—*Grammocephalus hybridatus*—n.sp —head, lateral view. (Orig.)

Fig. 208 (*a*), (*b*), (*c*).—*Grammocephalus hybridatus*—n.sp.—buccal teeth variations. (Orig.)

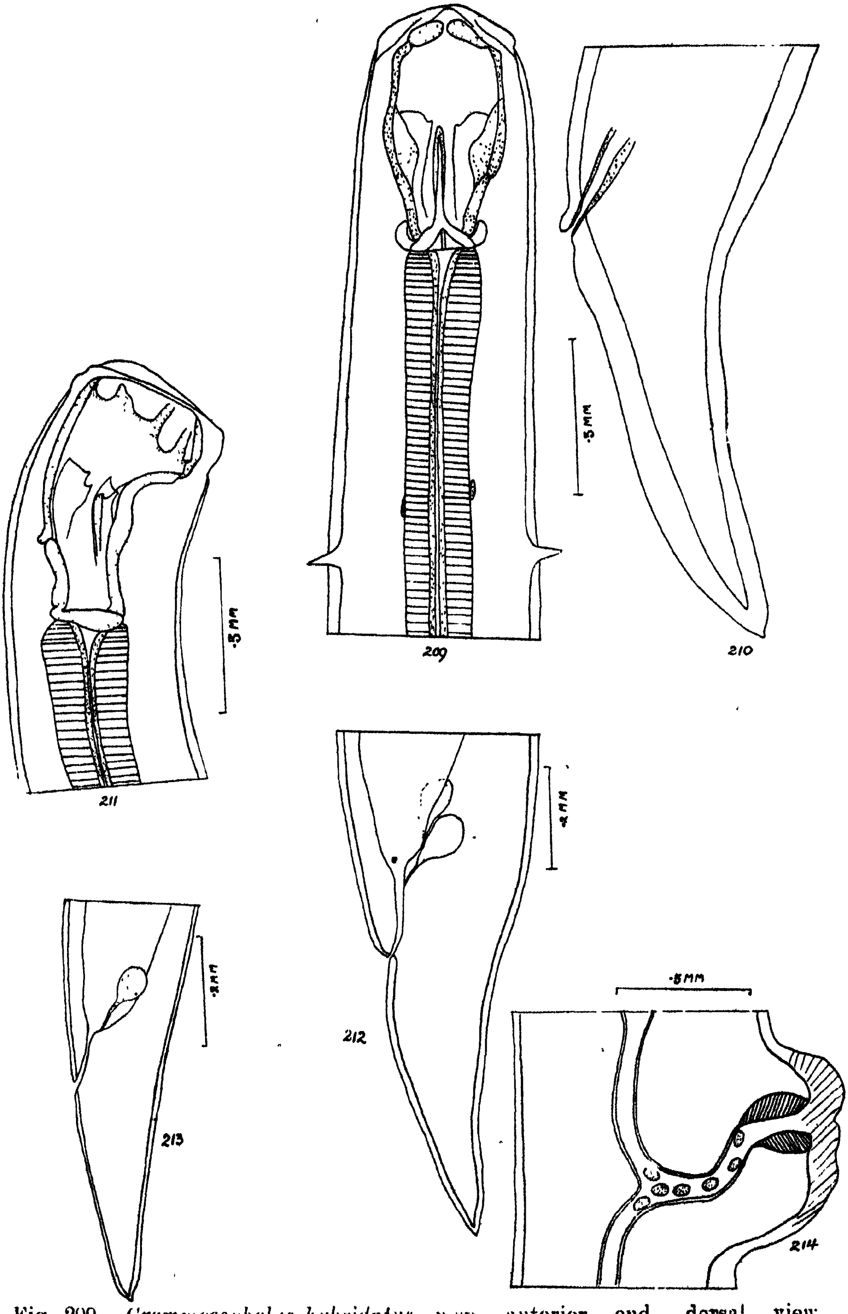


Fig. 209.—*Grammocephalus hybridatus*—n.sp.—anterior end, dorsal view. (Orig.)

Fig. 210.—*Grammocephalus hybridatus*—n.sp.—female tail. (Orig.)

Fig. 211.—*Grammocephalus hybridatus*—n.sp.—anterior end of larval form, lateral view. (Orig.)

Fig. 212.—*Grammocephalus hybridatus*—n.sp.—larval female tail. (Orig.)

Fig. 213.—*Grammocephalus hybridatus*—n.sp.—larval female tail. (Orig.)

Fig. 214.—*Grammocephalus hybridatus*—n.sp.—vulvar region of adult. (Orig.)

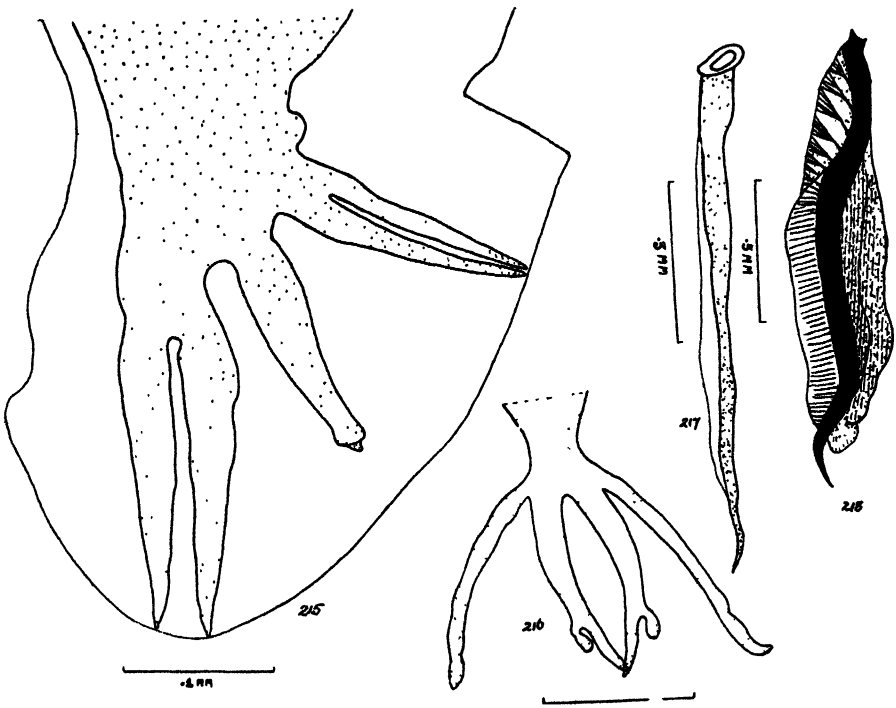
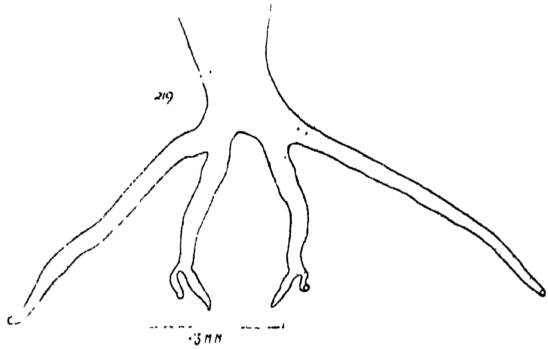


Fig. 215.—*Grammocephalus hybridatus*—n.sp.—larval male bursa, lateral view. (Orig.)

Fig. 216.—*Grammocephalus hybridatus*—n.sp.—larval male, dorsal ray. (Orig.)

Fig. 217.—*Grammocephalus hybridatus*—n.sp.—left spicule of larval male. (Orig.)

Fig. 218.—*Grammocephalus hybridatus*—n.sp.—right spicule of adult male. (Orig.)



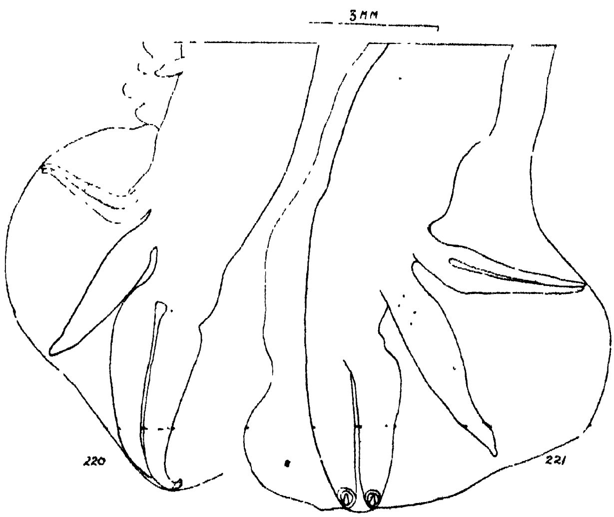
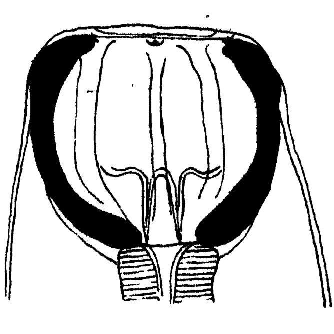


Fig. 219.—*Grammocephalus hybridatus*—n.sp.—dorsal ray of adult male. (Orig.)
Fig. 220.—*Grammocephalus hybridatus*—n.sp.—lateral lobe of bursa, external view. (Orig.)
Fig. 221.—*Grammocephalus hybridatus*—n.sp.—lateral lobe of bursa, inside view. (Orig.)



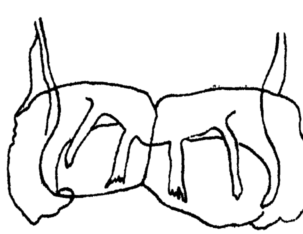


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Fig. 222.—*Syngamus indicus*—head, lateral view. (After Mönnig, 1932.)
Fig. 223.—*Syngamus indicus*—male bursa, dorsal view. (After Mönnig, 1932.)

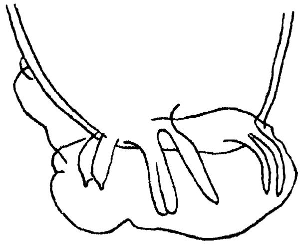

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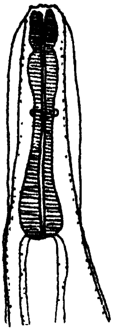
Fig. 224.—*Syngamus indicus*—male bursa, lateral view. (After Mönnig, 1932.)
Fig. 225.—*Syngamus indicus*—female tail. (After Mönnig, 1932.)



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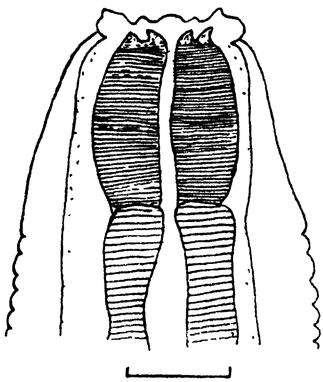

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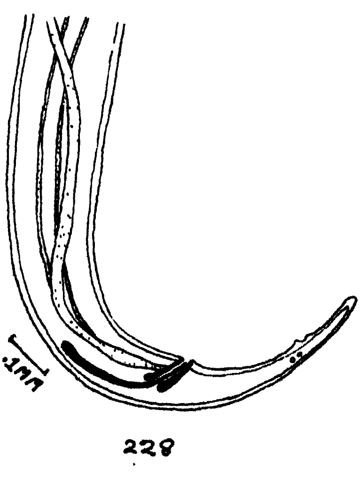
Fig. 226.—*Leiperenia leiperi*—anterior end. (After Khalil, 1922.)
Fig. 227.—*Leiperenia leiperi*—head. (After Khalil, 1922.)



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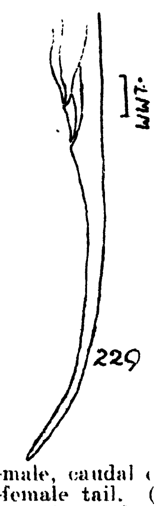

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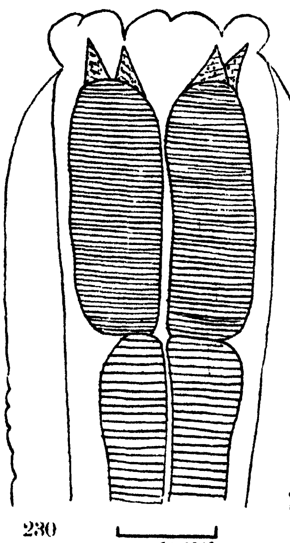

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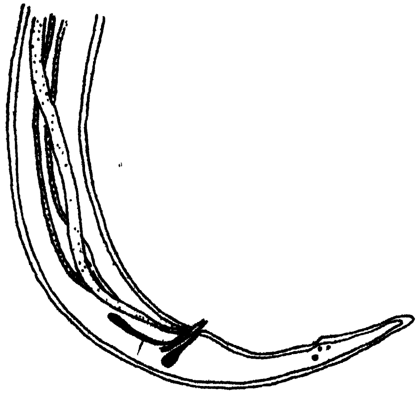
Fig. 228.—*Leiperenia leiperi*—male, caudal end. (After Khalil, 1922.)
Fig. 229.—*Leiperenia leiperi*—female tail. (After Khalil, 1922.)
Fig. 230.—*Leiperenia galebi*—anterior end. (After Khalil, 1922.)



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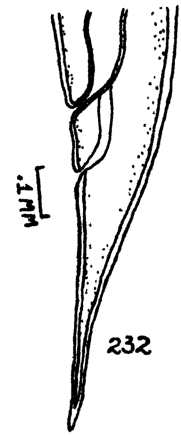

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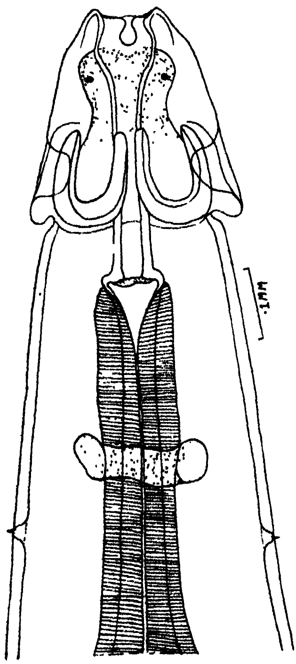
Fig. 231.—*Leiperenia galebi*—male, caudal end. (After Khalil, 1922.)
Fig. 232.—*Leiperenia galebi*—female tail. (After Khalil, 1922.)



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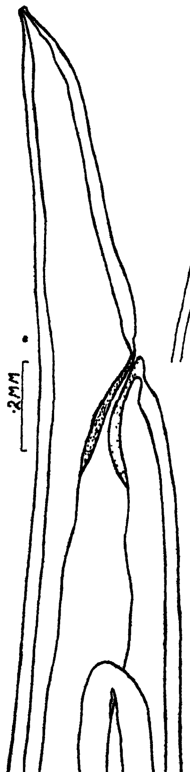

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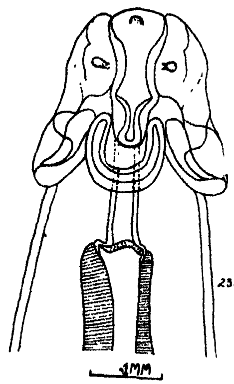

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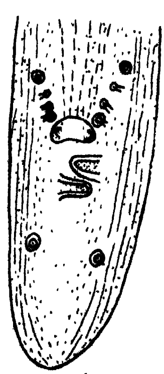

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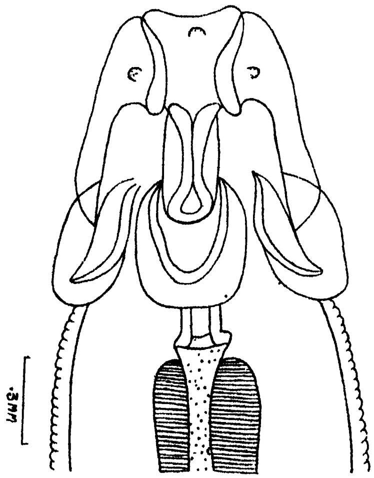
Fig. 233.—*Parabronema indicum*—anterior end, dorsal view. (Orig.)
Fig. 234.—*Parabronema indicum*—female tail. (Orig.)
Fig. 235.—*Parabronema indicum*—head, lateral view. (Orig.)
Fig. 236.—*Parabronema indicum*—male, caudal end, ventral view. (After Baylis, 1921.)



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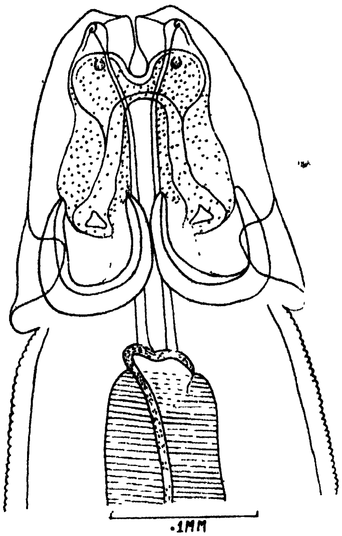

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Fig. 237.—*Parabronema smithii*—head, lateral view. (Orig.)
Fig. 238.—*Parabronema smithii*—head, dorsal view. (Orig.)

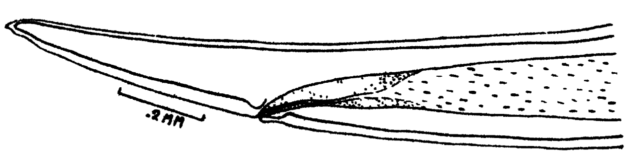

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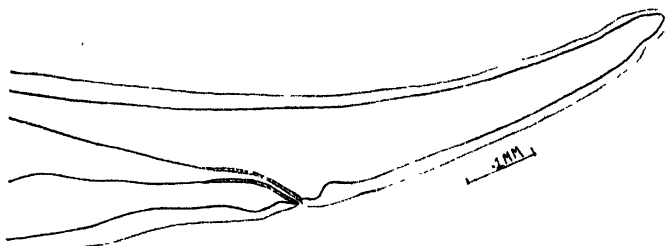
Fig. 239.—*Parabronema smithii*—female tail. (Orig.)



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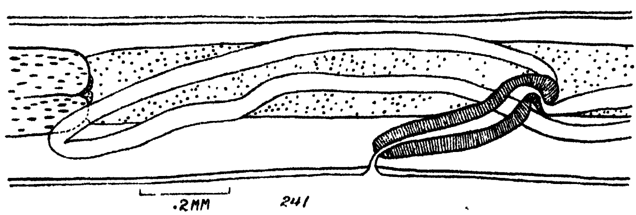

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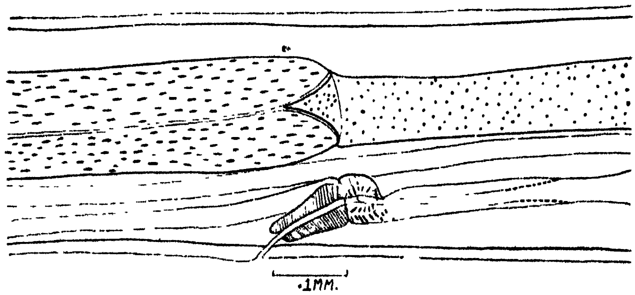

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Fig. 240.—*Parabronema smithii*—female tail. (Orig.)

Fig. 241.—*Parabronema smithii*—vulvar region. (Orig.)

Fig. 242.—*Parabronema smithii*—vulvar region. (Orig.)

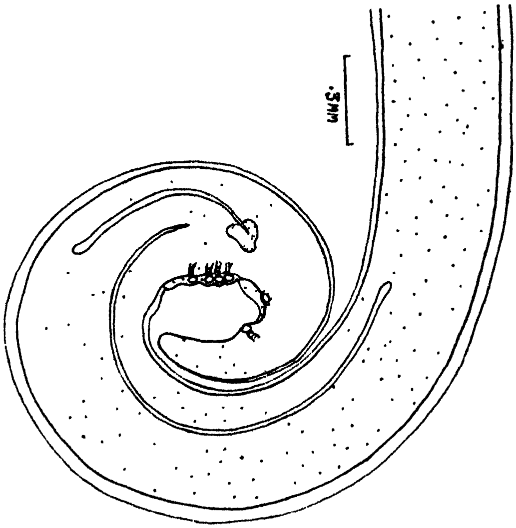
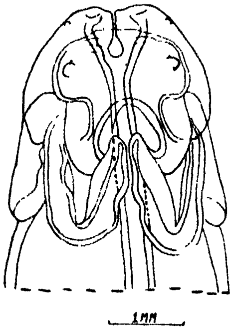


Fig. 243.—*Parabronema smithii*—male, caudal end, lateral view. (Orig.)



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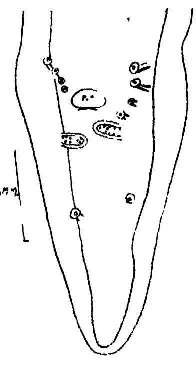

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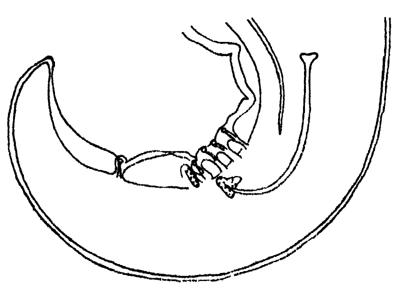

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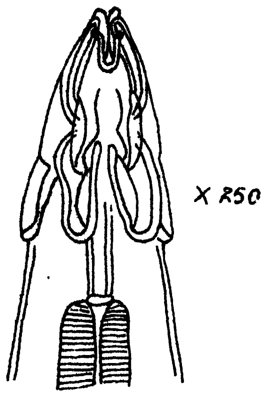
Fig. 244.—*Parabronema africanum*—head, dorsal view. (After Baylis, 1921.)
Fig. 245.—*Parabronema africanum*—hale, caudal end, ventral view . (After Baylis, 1921.)
Fig. 246.—*Parabronema africanum*—male, caudal end, lateral view. (After Baylis, 1921.)



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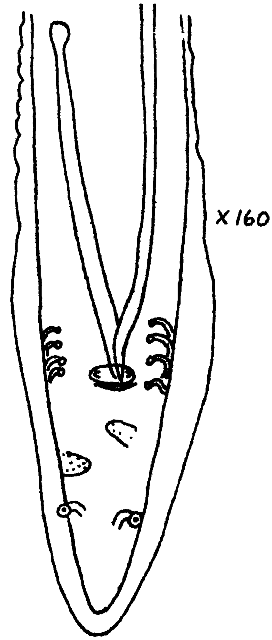

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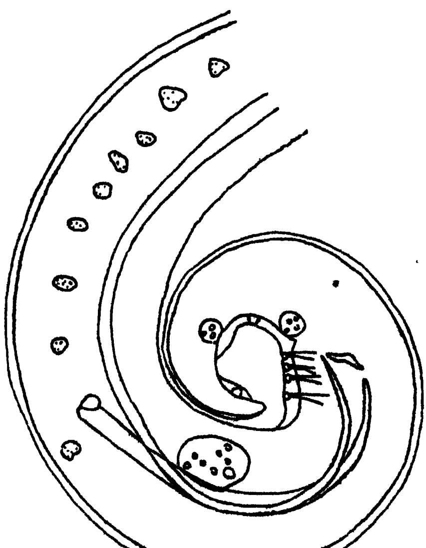

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Fig. 247.—*Parabronema rhodesiense*—anterior end. (After York & Maplestone, 1926.)

Fig. 248.—*Parabronema rhodesiense*—male, caudal end, ventral view. (After York & Maplestone, 1926.)

Fig. 249.—*Parabronema rhodesiense*—male, caudal end, lateral view. (After York & Maplestone, 1926.)

ABBREVIATIONS.

c. = cirrus sac.
c.f. = caudal flap.
c.p. = cirrus pouch.
c.s. = cirrus sac.
e.p. = excretory pore.
e.v. = excretory vesicle.
g.p. = genital pore.
g. pap. = genital papilla.
g.s. = genital sucker.
h.b. = hermaphroditic bulb.
h.d. = hermaphroditic duct.
i.c. = intestinal caeca.
L.c. = Laurer's canal.
m. = musculosa.
o.b. = oesophageal bulb.
o.d. = oral diverticulum.
oes. = oesophagus.
o.s. = oral sucker.
ov. = ovary.
p.p. = pars prostatica.
p.s. = posterior sucker.
s.b. = suctorial bulb.
sh. gl. = shellgland.
s.p. = suctorial pouch.
s.v. (v.s.) = seminal vesicle.
t. = testes.
ut. = uterus.
vit. = vitellaria.

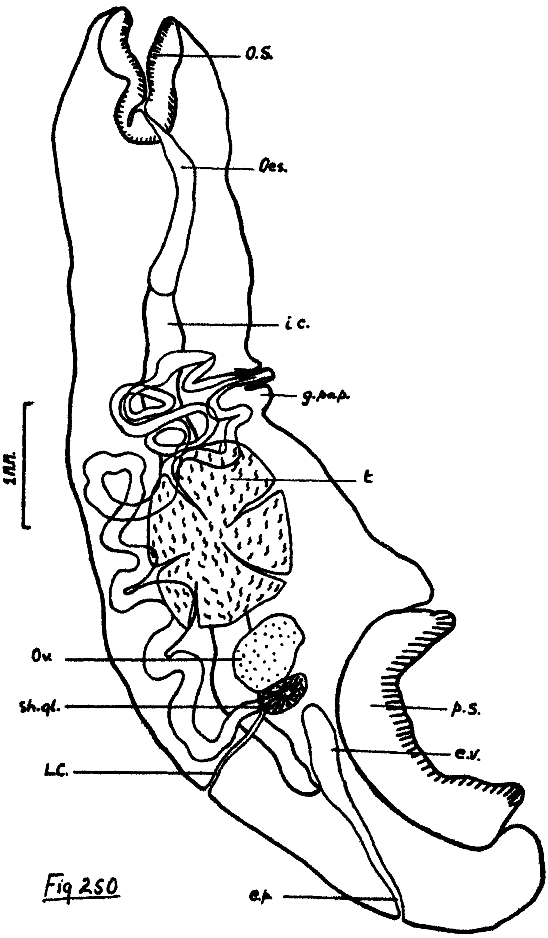
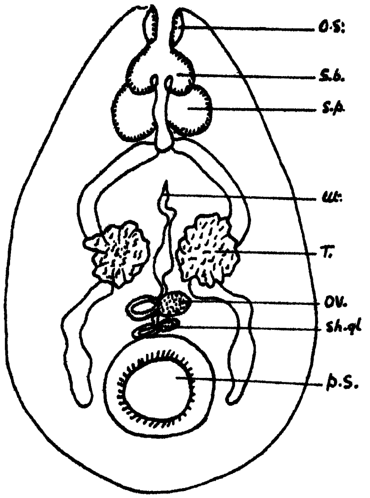


Fig. 250.—*Pseudodiscus collinsi*—sagittal section. (Orig.)



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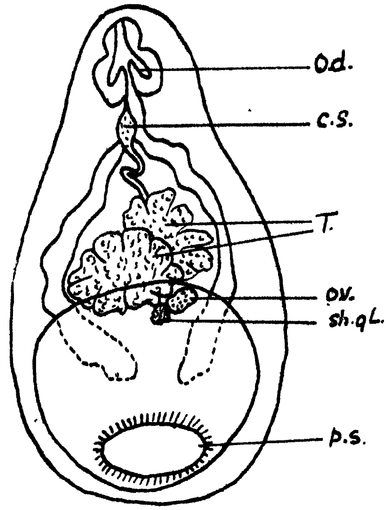

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Fig. 251.—*Pseudodiscus collinsi*—(From Travassos, 1934, after Stiles & Goldberger, 1910.)

Fig. 253.—*Pseudodiscus hawkesii*—(From Travassos, 1934, after Stiles & Goldberger, 1910.)

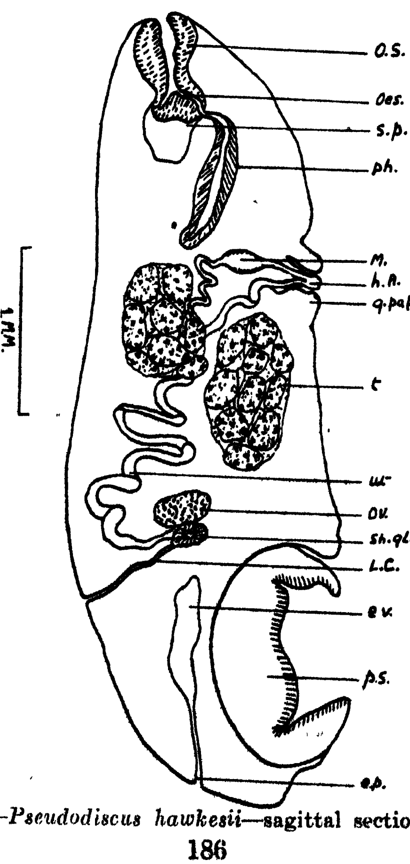
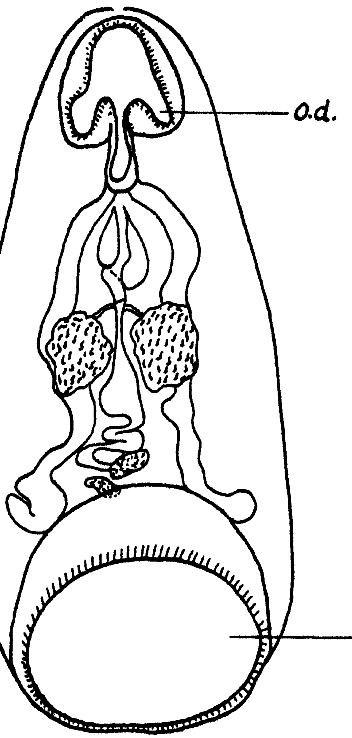


Fig. 252.—*Pseudodiscus hawkesii*—sagittal section. (Orig.)



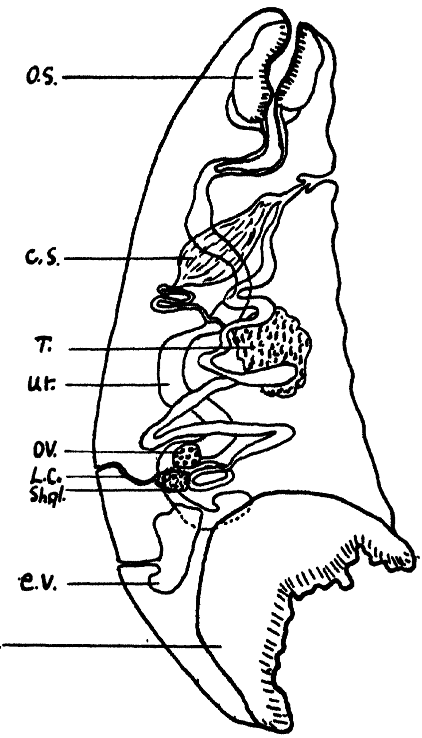
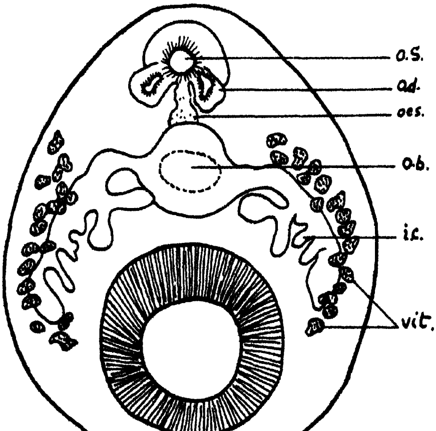


Fig. 254.—*Pfenderius papillatus*—(From Travassos, 1934, after Stiles & Goldberger, 1910.)

Fig. 255.—*Pfenderius papillatus*—sagittal section. (From Travassos, 1934, after Stiles & Goldberger, 1910.)



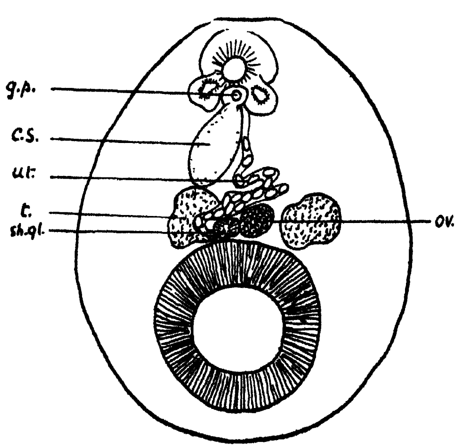
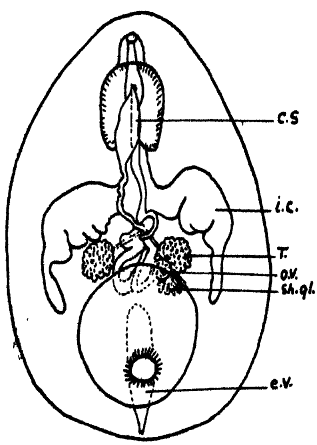


Fig. 256.—*Pfenderius birmanicus*—(After Bhalerao, 1935.)
Fig. 257.—*Pfenderius birmanicus*—(After Bhalerao, 1935.)



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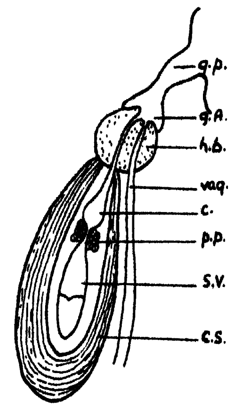

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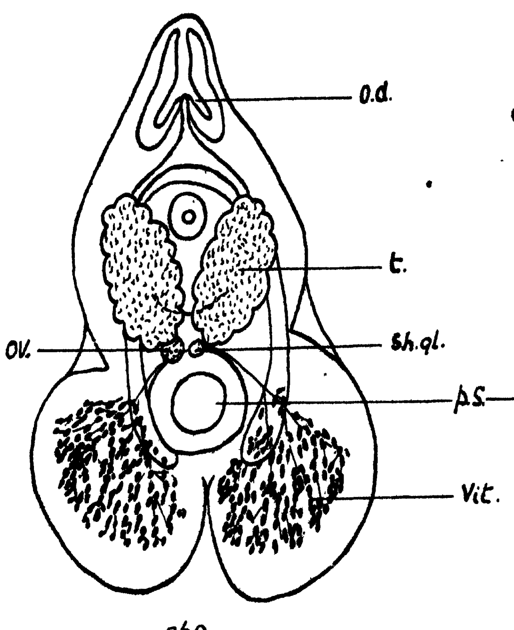
Fig. 258.—*Pfenderius heterocaeca*—(From Trav., 1934, after Fukui, 1926.)

Fig. 259.—*Pfenderius heterocaeca*—cirrus sac, and terminal portion of genitalia. (After Bhalerao, 1935.)



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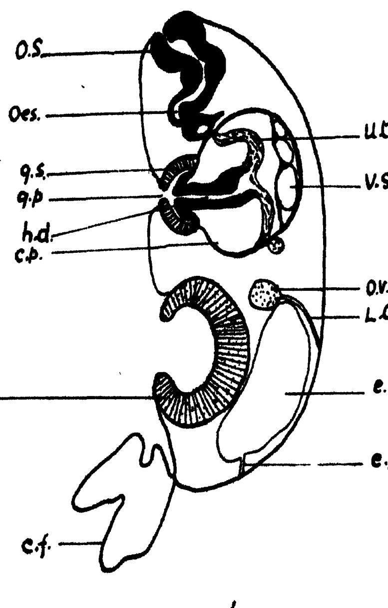

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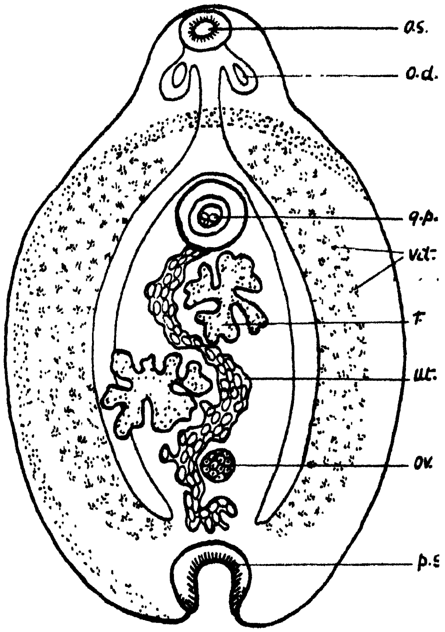
Fig. 260.—*Brumptia bicaudata*—(After Trav., 1934.)

Fig. 261.—*Brumptia bicaudata*—sagittal section. (After Maplestone, 1923.)



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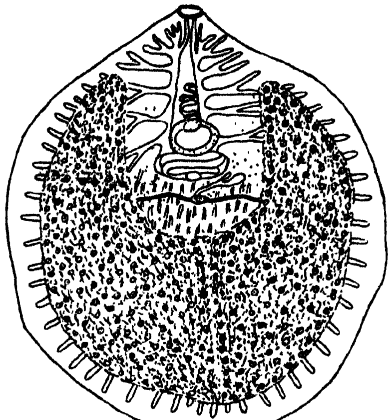

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Fig. 262.—*Gastrodiscus secundus*×10.—ventral view. (After Bhalerao, 1931.)
Fig. 263.—*Fasciola jacksoni*—(After Evans & Rennie, 1909.)

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Fig. 264.—*Anoplocephala manubriata*—scolex, dorsal view. (After Raill., Henry & Bauche, 1914.)

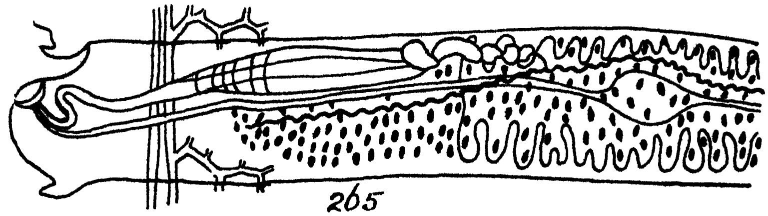

Fig. 265.—*Anoplocephala manubriata*—marginal portion of segment, dorsal view.

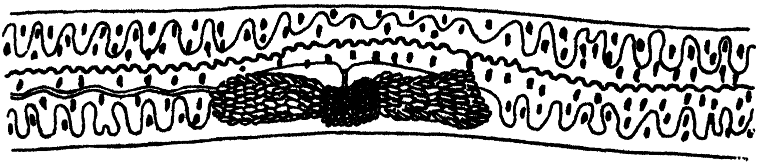

Fig. 266.—*Anoplocephala manubriata*—median portion of segment, dorsal view.

Section III.

Mineral Metabolism and Nutrition.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 10, *Number* 1, *January*, 1938.

Printed in the Union of South Africa by the Government Printer, Pretoria.

Protein Studies.

Plant Proteins I.—A Comparative Study of the Growth-promoting Properties of the Proteins of Peanutmeal, Sesamemeal, Coprameal, Lucernemeal, and Cottonseedmeal.

By D. B. SMUTS, Section of Biochemistry and Nutrition, Onderstepoort.

IN the course of extensive investigations at this Institute on the mineral deficiencies prevailing under natural conditions of grazing, indications pointing to a total or partial protein deficiency during certain seasons of the year have been observed. This observation, together with the fact that there exists a complete lack of reliable data on the constitution, digestibility, utilization, and nutritive values of our protein feeds in general, which seriously impair any attempt at compounding rations or supplementing protein deficient grazing on a scientific and economical basis, gave rise to the initiation at this Institute of a fairly extensive programme covering the various aspects of protein nutrition.

The problem of protein requirements, although an extremely important phase of animal nutrition, has on account of its complex nature so far defied almost every attempt at a complete and systematic solution. The method of expressing protein requirements on the basis of digestible protein, as is the popular procedure, is a survival of the times when chemical differences in the constitution of proteins were not appreciated. Nevertheless, this method of expressing protein requirements has been of immense practical value in the past and will probably continue in its popularity for the time being in lieu of a more scientific and appropriate unit of expression. However, it is fairly clear that the digestible protein conception is an expression without any physiological significance or interpretation in that it is entirely based on the difference between nitrogen intake and nitrogen evacuated in the faeces. Consequently it affords no indication as to the manner or amount of protein utilized by the animal

or the quantity necessary for maintaining the integrity of the tissues. It has also been amply demonstrated that protein feeds or mixtures of such feeds differ widely in their nutritive values, and that these differences are directly attributable to the different proportions and amounts of amino acids present in the respective protein molecules. Expressing, therefore, the requirements of protein in terms of digestibility irrespective of the differences in the amino acid constitution is not only a misrepresentation of the actual biological value of such proteins but also incongruous with the present conception on which the metabolism of proteins is based.

It appears, therefore, that an entirely new line of investigation should be followed in order to arrive at a means of expressing protein requirements, which would not only be applicable in practice but also respects the laws governing protein metabolism. Such a method has been proposed by Mitchell (1926), who recognised two distinct phases in the problem of determining the protein requirements of animals. Firstly the determination of protein requirements in terms relating to animal tissues and animal products, for the preservation and elaboration of which food protein is needed and, secondly, the determination of protein values of farm feeds and mixture of feeds to cover these requirements. According to Mitchell these two problems, although closely connected, must be investigated separately and by different means in order to arrive at a complete solution. The former may be measured by the total nitrogen content of the tissue constituents catabolized endogenously (maintenance) or by the total nitrogen content of the new tissues formed in growth and reproduction or by the total nitrogen content of the milk produced in lactation. A measurement of the latter component must consider the total protein content of the food or ration, the loss or wastage of protein in digestion and the loss or wastage of protein in the process of its conversion into tissue constituents or the constituents of body secretions. If, therefore, ultimately by this method the amount of protein utilized for the elaboration of new tissue, for instance, and on the other hand the biological value of a protein feed for growth be determined, it becomes a simple matter to state or calculate the quantity of digestible crude protein necessary to cover the protein requirements for growth. In a similar manner the protein requirements for the other forms of production may be calculated.

Thus far a general application of this method of expressing the protein requirements of animals has been rendered impossible through the lack of sufficient data. It is the intention in this programme of protein investigation to apply this method throughout and accumulate sufficient data for its practical application. In order to approach the problem systematically it was decided to concentrate investigations at first entirely on the second phase, namely, the determination of the protein values of farm feeds and mixture of feeds for the different requirements of farm animals. These investigations are first carried out with rats and later extended to include the different types of farm animals. Such a study would necessarily include: (1) a comparison of the nutritive values of the proteins of feeds or mixtures of foods; (2) the biological value of the proteins of such feeds; (3) the supplementary effect amongst their proteins; (4) the

amino acid deficiencies limiting the proper utilization of the proteins of feeds or combination of feeds. It will be appreciated that once this information is available it will be possible for practical purposes to select protein feeds or mixture of feeds of the highest biological values.

Although it is generally recognised that protein feeds differ distinctly in nutritive value, very few comparisons have actually been carried out in which food intake was controlled and the results treated statistically. The omission of these two factors in experiments of this nature detracts greatly from their significance. Mitchell and Beadles (1930), Braman (1931), Haag (1931), and Mitchell and Smuts (1932) have found the paired feeding method, developed by Mitchell (1930), very efficient in measuring the nutritive differences between protein feeds as well as their amino acid deficiencies. Braman (1931), applying this method, ascertained that cottonseedmeal is inferior in nutritive value to linseedmeal. However, their biological values were almost identical, the difference detected in the growth studies being due probably to the lower digestibility of the protein of cottonseedmeal. In pigs, Mitchell and Hamilton (1931) were able to substantiate the results of Braman and found no difference between the biological values of cottonseedmeal and linseedmeal. Nevens (1921), in comparing the proteins of cottonseedmeal and lucerne, found that the former is superior to the latter. Richardson and Green (1917) state that cottonseedmeal and flour are satisfactory sources of protein for the growth of oats. Bethke and co-workers (1928), using the method of Osborne and Mendel with rats, found no difference between the protein of linseedmeal and cottonseedmeal. With beef calves they could not detect any measurable difference when cottonseedmeal and linseedmeal were fed in combination with lucerne-hay.

Haag (1934), in a modification of the paired feeding method, found that the protein of bran was superior to that of lucerne. Morris and Wright (1933), in a determination of the relative efficiencies of protein feeds for milk production, found that decorticated earthnut cake was inferior to bloodmeal, peameal and beanmeal, but slightly better than linseedmeal. Schundt (1934) and co-workers obtained a higher nitrogen retention with yeast protein than with peanut in pigs, the nitrogen retention of peanut being higher than that of soyabeans. Daniels and Loughlin (1918) conclude that peanut contains a good quality protein. Mitchell and Villegas (1923) in their work on cocoanutmeal and soyabeans found fit to state that at 5 per cent. level there was no distinct difference in these two proteins. However, at 10 per cent. level the proteins of cocoanutmeal are slightly but distinctly less effective than the proteins of soyabeans for structural purposes in the body of the rat.

Experimental.

Simultaneously with the determination of the biological values, comparative growth studies of the different protein feeds, based on the paired feeding method, were also conducted. Data referring to

the latter are presented in this paper. Rats were paired according to age, sex, weight and litter. Each pair received the same management and was kept under identical conditions, the only difference being the source of protein incorporated in the respective rations. Food was equated by the method of Mitchell (1930), namely, that in each pair both rats were given the same amount of food at the start and thereafter the daily amounts were regulated by the member of the pair eating the least. Rats were weighed weekly; the initial and final weights being the average weights of three consecutive days.

TABLE I.

Composition of Rations.

Peanut Meal	15·7	—	—	—	—
Lucerne Meal	—	57·5	—	–	—
Sesame Meal	—	—	25·0	—	—
Copra Meal	—	—	—	33·0	—
Cottonseed Meal	—	—	—	—	22·5
Sucrose	10·0	10·0	10·0	10·0	10·0
Butterfat	8·0	8·0	8·0	8·0	8·0
Yeast Extract (1)	10·0	10·0	10·0	10·0	10·0
Agar (2)	14·0	—	—	—	—
Cod Liver Oil	2·0	2·0	2·0	2·0	2·0
NacCI	1·0	1·0	1·0	1·0	1·0
Salt Mixture (3)	4·5	4·5	4·5	4·5	4·5
Starch	34·8	7·0	39·5	31·5	42·0
TOTALS	100·0	100·0	100·0	100·0	100·0
Per cent. Nitrogen	1·45	1·51	1·51	1·56	1·54

(1) Yeast extract was prepared according to the method of Itter S, Orent E. R., and McCallum Ev. *J.C.B.*, Vol. 108, No. 2, pp. 571–577, 1935.

(2) Agar was added to peanut ration only in the comparison with lucerne meal, in all other cases it was displaced by an equal weight of starch.

(3) A modified Osborne and Mendel Mixture described by P. B. Hawk and B. L. Oser, 1931. *Science* Vol. 74, p.369.

The composition by weight of the rations is given in Table I. Every ration was analysed for total nitrogen before the start of the experiment, in order to equalize the nitrogen content of the rations as nearly as possible either by remixing or adding more of the proteins under experiment or by making up fresh batches of food. In this manner close agreement between the nitrogen content of the different rations could be obtained. All the protein feeds except oats, which was a product prepared by a commercial company as a breakfast food, were similar to those used under practical feeding conditions except that they were put through a mill and reduced to a fine powder which facilitated mixing and ensured a more homogeneous ration.

TABLE II.

Summary of Bodyweights, Gains, Food Records and Number of Refusals (in grms.).

TRIPLICATE...	1.			2.			3.			4.			5.			6.		
	Lucerne.	Lucerne and Peanut.	Peanut.	Lucerne.	Lucerne and Peanut.	Peanut.	Lucerne.	Lucerne and Peanut.	Peanut.	Lucerne.	Lucerne and Peanut.	Peanut.	Lucerne.	Lucerne and Peanut.	Peanut.	Lucerne.	Lucerne and Peanut	Peanut.
Initial Weight.....	62·0	65·0	64·5	57·5	62·5	60·5	60·0	62·0	64·0	57·0	57·0	55·0	50·5	56·0	52·0	48·0	55·0	54·0
Final Weight......	92·0	101·0	100·0	87·0	92·0	90·0	88·0	92·0	100·0	82·0	91·0	93·0	70·0	68·0	76·0	75·0	84·0	84·5
Gains.............	30·0	36·0	35·5	29·5	29·5	29·5	28·0	30·0	34·0	25·0	34·0	38·0	19·5	12·0	24·0	27·0	29·0	30·5
Food Consumption	353	353	353	334	334	334	331	331	331	330	330	330	321	321	321	280	280	280
Refusals..........	3	1	6	1	2	13	2	2	6	1	0	3	0	0	9	0	5	0

Results.

In Table II is given a summary of the comparisons between lucernemeal, lucernemeal plus peanut and peanutmeal. As will be noticed the test was a modification of the paired feeding method in that triplicates instead of pairs were used. The conditions of feeding and pairing of rats were identical to those practised under paired feeding. The amount of food consumed during the experimental period was of the same magnitude for each member of the triplicate. Rats receiving the peanut ration in most cases limited the food consumption of their mates by refusing part of their daily feed when the latter was increased above a certain level. This was probably due, as Mitchell suggested, to the unpalatability of the peanutmeal.

Due to the layout of the experiment, it is statistically incorrect to compare one treatment like lucernemeal with another (peanutmeal) before the significance of the test as planned is ascertained. The mean gain in weight for the different treatments over the experimental period is 26·5 grams for lucernemeal, 28·4 grams for lucerne plus peanut, and 31·9 grams for peanutmeal. The standard error of the mean equals $\pm 2{\cdot}096$. By an analysis of the variance it is evident that the variations between treatments are by no means signficantly greater than the remainder variance. Such an outcome would naturally imply that there is no reason to deduce from the data under observation, that there was a difference in response ascribable to the different treatments. When, however, the lucerne plus peanut ration is entirely ignored and lucernemeal is compared with peanutmeal the difference between these two treatments becomes significant. The mean difference (m) in grams is equal to $5{\cdot}417 \pm 1{\cdot}7485$ gms.; t is equal to $\dfrac{m}{sz \text{ of mean}} = 3{\cdot}098$ and the degrees of freedom $(n-1) = 5$. Under these conditions the probability

Table III.

A Comparison of the Weekly Gains between Peanut and Lucernemeal.

Weeks.	Pair 1.	Pair 2.	Pair 3.	Pair 4.	Pair 5.	Pair 6.	Totals per Week. +	−	±
1	+	+	+	+	+	+	6	0	0
2	+	+	+	+	+	−	5	1	0
3	+	+	+	+	−	±	4	1	1
4	+	−	−	+	−	+	3	3	0
5	−	−	+	−	+	±	2	3	1
6	+	−	−	+	−	+	3	3	0
						Total for Experiment	23	11	2

+ Indicates greater gain by Peanut Meal.
− Indicates greater gain by Lucerne Meal.
± Indicates equal gain.

(P) that the difference in favour of peanut is not a chance effect is $0 \cdot 03$. Since a value of $P = 0 \cdot 05$ is in current biometrical practice a criterion of significance, it is obvious that peanut, by this method of comparison, is superior to lucernemeal. But in view of the actual lay-out of the experiment this significance, when judged by the entire data, must be considered accidental (Probability approximately 11 per cent.).

A comparison of weekly gains is ordinarily a less reliable and legitimate method of comparison than that of total gains, due to the fact that weekly food residues may appreciably affect the gains. The necessary conditions for statistical independence between individual values are also seriously infringed in that the weekly gains for the same pair of rats are not independent in so far as they reflect an inherent difference between the two rats. If such inherent differences exist the weekly gains are not comparable on a basis of random variation on the assumption that each rat in any one pair has, except for treatment differences, an equal chance to gain more than its mate during a particular week. Hence, due to this lack of independence in weekly gains for the same pair of rats, it is not quite correct to consider the product of the number of pairs and weeks as the total number of degrees of freedom.

Furthermore, the number of weekly gains is by no means independent of the total gains. If a particular rat of one pair gains more in weight than its weight during the experimental period, it is likely that it should have gained more in weight during the greater number of weeks. Hence the two comparisons, total gains and number of greater weekly gains, are by no means independent, but to some extent one and the same comparison. Consequently it is doubtful whether any extra information is gained by comparing weekly gains after an analysis of total gains has been made. The inclusion of comparisons between weekly gains in this paper is merely by reason of its adoption by other workers and to illustrate what was already shown by the analysis of total gains.

In a comparison of lucernemeal and peanutmeal the weekly food intake was the same for the different triplicates. This naturally gives a greater value to the statistical analysis of the weekly gains. If the number of equal weekly gains is divided equally between peanut and lucernemeal, it will be seen that out of the 35 comparisons 24 favoured peanutmeal and only 12 lucernemeal. This fact also points to a superiority of peanutmeal over lucernemeal.

In Table IV the data pertaining to the comparison of peanutmeal and sesamemeal are tabulated. Here, again, it is obvious from the number of refusals that the peanut fed rat in all but one case was the one to limit the food intake of the respective pairs. A statistical analysis of the total gains shows that the mean difference of the six pairs of rats is zero and, consequently, there is no indication whatsoever of an existing difference between the protein of peanutmeal and sesamemeal. A comparison of the weekly gains in Table V affords confirmatory evidence as to the equality of these two protein feeds. If chance alone operated, it would be expected that of the 36 comparisons the ideal outcome would be 18 for both treatments.

Table IV.

Summary of Bodyweights, Gains, Feed Records and Refusals on Peanut and Sesamemeal Rations (in gms.).

	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Peanut Meal.	Sesame Meal.	Peanut Meal.	Sesame Meal.	Peanut Meal.	Sesame Meal.	Peanut Meal.	Sesame Meal.	Peanut Meal.	Sesame Meal.	Peanut Meal.	Sesame Meal.
Initial Weight	85·0	82·0	77·0	77·0	74·0	77·0	87·0	85·0	94·0	94·0	70·0	74·0
Final Weight	131·0	129·0	128·0	124·0	120·0	121·0	134·0	129·0	132·0	137·0	110·0	117·0
Gain gms	46·0	47·0	51·0	47·0	46·0	44·0	47·0	44·0	38·0	43·0	40·0	43·0
Total Food Consumption	361	361	369	369	364	364	394	394	386	386	351	351
Refusals Period	3	1	1	3	6	0	4	0	2	0	5	0

When the equal gains are divided equally between peanut and sesamemeal, the respective numbers of weekly gains become 16·5 for sesamemeal and 19·5 for peanut. These figures very nearly approach the ideal outcome and therefore confirm the non-existing difference in the two treatments.

TABLE V.

A Comparison of Weekly Gains between Peanutmeal and Sesamemeal.

Weeks.	Pair 1.	Pair 2.	Pair 3.	Pair 4.	Pair 5.	Pair 6.	Totals per Week. +	Totals per Week. −	Totals per Week. ±
1	−	−	−	−	−	−	0	6	0
2	+	−	+	+	−	−	3	3	0
3	−	+	±	+	+	−	3	2	1
4	+	+	±	±	−	+	3	1	2
5	+	−	+	−	+	+	4	2	0
6	−	+	−	+	−	−	2	4	0
TOTAL EXPERIMENT							15	18	3

\+ Indicates greater gain by Peanut Meal.
− Indicates greater gain by Sesame Meal.
± Indicates equal gain.

In a comparison of peanutmeal and coprameal in Table VI, it is fairly clear by inspecting the total gains of the different pairs, that no obvious difference exists as a result of the different treatments. The mean difference in total gain is $0 \cdot 333 \pm 1 \cdot 837$ grams, showing that the mean difference is much less than its standard error. The value of $t = 0 \cdot 164$ is insignificant and the probability that the observed difference in favour of peanutmeal is due to chance alone is approximately 89 per cent. In a comparison of the weekly gains, Table VII, the deviation of the two treatments from the ideal outcome of 18, when the equal gains are allotted proportionately to peanut and coprameal, is so small that it becomes entirely insignificant. It is, therefore, safe to conclude that coprameal and peanutmeal do not differ significantly in nutritive value.

TABLE VII.

Comparisons of Weekly Gain between Peanutmeal and Coprameal.

Weeks.	Pair 1.	Pair 2.	Pair 3.	Pair 4.	Pair 5.	Pair 6.	Totals per Week. +	Totals per Week. −	Totals per Week. ±
1	+	±	−	+	−	−	2	3	1
2	+	−	±	−	+	−	2	3	1
3	+	+	+	−	±	−	3	2	1
4	±	+	+	−	−	+	3	2	1
5	±	+	−	−	+	−	2	3	1
6	+	−	+	+	−	+	4	2	0
TOTAL EXPERIMENT							16	15	5

\+ Indicates greater gain by Peanut Meal.
− Indicates greater gain by Copra Meal.
± Indicates equal gain.

Table VI.

Summary of Bodyweights, Gains, Feed Records and Refusals on Peanut and Coprameal Rations (in gms.).

	Pair 1.		Pair 2.		Pair 3.		Pair 4.		Pair 5.		Pair 6.	
	Peanut Meal.	Copra Meal.	Peanut Meal.	Copra Meal.	Peanut Meal.	Copra Meal.	Peanut Meal.	Copra Meal.	Peanut Meal.	Copra Meal.	Peanut Meal.	Copra Meal.
Initial Weight	99·0	99·0	92·0	93·0	85·0	85·0	83·0	84·0	77·0	77·0	87·0	80·0
Final Weight	132·0	126·0	126·0	127·0	121	126·0	118·0	118·0	126·0	114·0	112·0	111·0
Gains gms	33·0	27·0	34·0	34·0	36·0	41·0	35·0	34·0	49·0	37·0	25·0	31·0
Total Food Consumption	341	341	338	338	343	343	311	311	329	329	315	315
Refusals	4	0	.3	0	1	5	4	6	4	2	5	5

Table VIII.

Summary of Bodyweights, Gains, Feed Records and Refusals on Peanut and Cottonseedmeal Rations (in gms.).

	Peanut Meal.	Cotton-seed Meal.	Peanut Meal.	Cotton-seed Meal.	Peanut Meal.	Cotton-seed Meal.	Peanut Meal.	Cotton-seed Meal.	Peanut Meal.	Cotton-seed Meal.	Peanut Meal.	Cotton-seed Meal.
Initial Weight, gms	90·0	85·0	114·0	114·0	98·0	95·0	87·0	87·0	92·0	94·0	87·0	89·0
Final Weight, gms	120·0	122·0	140·0	149·0	125·0	135·0	120·0	117·0	126·0	135·0	125·0	131·0
Gain, gms	30·0	37·0	26·0	35·0	27·0	40·0	33·0	30·0	34·0	41·0	38·0	42·0
Total Food Consumption	343	343	372	372	339	339	300	300	352	352	378	378
Refusals	5	1	1	1	0	0	5	0	4	0	0	2

That cottonseedmeal seems to be superior to peanutmeal is indicated by the fact that five out of the six pairs of rats show a bigger total gain in favour of cottonseedmeal. Analysing the total gains in weight (Table VIII), it is found that the mean difference for the six pairs is $m = 6 \cdot 17 \pm 2 \cdot 191$ grams, and t becomes equal to $2 \cdot 808$. The possibility, therefore, that the above difference in favour of cottonseedmeal is due to chance alone is $0 \cdot 04$. This probability is so small, and less than 5 per cent. which is usually taken as the limit of significance, that it may be concluded that cottonseedmeal is superior to peanutmeal. As no difference was demonstrated between peanutmeal, coprameal, and sesamemeal, it seems reasonable to deduce that cottonseedmeal is also superior to the latter two meals.

TABLE IX.

Comparison of Weekly Gains made by Pair-mates on Peanutmeal and Cottonseedmeal.

Weeks.	Pair 1.	Pair 2.	Pair 3.	Pair 4.	Pair 5.	Pair 6.	Totals per Week.		
							+	−	±
1	−	−	−	+	+	−	2	4	0
2	+	±	−	+	±	±	2	1	3
3	±	−	−	−	+	±	1	3	2
4	−	−	+	−	−	−	1	5	0
5	−	±	−	±	−	−	0	4	2
6	±	−	−	±	−	−	0	4	2
TOTAL EXPERIMENT							6	21	9

\+ Indicates greater gain by Peanut Meal.
— Indicates greater gain by Cotton-seed Meal.
± Indicates equal gain.

A comparison of the weekly gains (Table IX) shows six comparisons favouring the peanut fed rat, twenty-one favouring cottonseedmeal and nine equal gains. If the latter is divided equally amongst the peanut and cottonseed fed rats, it is found that $10 \cdot 5$ of the comparisons favour peanut and 25.5 favour cottonseedmeal, which means a deviation of $7 \cdot 5$ from the ideal outcome. The standard deviation of the frequency distribution of the outcome of 36 events, each of which may result with equal probability either one of two ways is given by the expression $\sqrt{0 \cdot 5 \times 0 \cdot 5 \times 36}$, which is equal to $3 \cdot 0$. The deviation of $7 \cdot 5$ from the ideal chance outcome is, therefore, $2 \cdot 5$ times the standard deviation. Interpreting this ratio from the table of values of the normal probability integral, the probability exists that chance alone would produce this deviation approximately once in a hundred trials. This comparison, therefore, definitely supports the results on total gains, namely, that cottonseedmeal is a superior protein feed to peanutmeal.

While it appears from this investigation that cottonseedmeal is a better protein feed than coprameal, peanutmeal or sesamemeal, it does not exclude the possibility that these feeds may react distinctly different in other types of farm animals. This point is illustrated by the fact that Morris and Wright report that peanutmeal is slightly superior to linseedmeal for milk production, whereas Mitchell and Hamilton, as well as Braman, found no difference between cottonseedmeal and linseedmeal for growth and maintenance of the rat and the pig. In this study again cottonseedmeal has been found to be superior to peanutmeal in the rat. By inference it seems only natural to conclude that linseedmeal should be a better protein feed for growth than peanutmeal, which has been shown to be probably not the case for milk production. The value of protein feeds can, therefore, not be estimated or assumed at random from any set of data, but must be determined with different animals for each specific purpose. This phase of work is anticipated as investigations are extended to other animals. The superiority of cottonseedmeal over the protein feeds does not imply a general application in practice without taking into account the level of feeding at which this food may be toxic to certain species of animals.

Conclusion.

By means of the paired feeding method on rats it has been shown statistically that the protein of cottonseedmeal is superior to that of peanutmeal, while on the other hand no statistical difference could be detected between the proteins of peanutmeal and coprameal, and peanutmeal and sesamemeal. On this basis it seems reasonable to deduce that the proteins of cottonseedmeal are superior to sesame and coprameal. Evidence is presented which seems to suggest that lucernemeal is inferior to peanutmeal as a source of protein.

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Protein Studies.

Plant Proteins II.—The Biological Values of Lucernemeal Sesamemeal, Peanutmeal, Coprameal, Cottonseedmeal, and Oatmeal.

By D. B. SMUTS and A. I. MALAN, Section of Biochemistry and Nutrition, Onderstepoort.

THE conception of biological values of proteins was first introduced by Thomas, who defined biological value as the number of parts of body nitrogen replaced by 100 parts of food protein. His technique, adapted to human beings, was subsequently modified and elaborated by Mitchell (1924) and Boas Fixsen and Jackson (1932) for application to rats, and forms at present the basis of measuring the biological value of proteins by the nitrogen balance sheet method. Other methods of estimating the biological or nutritive values of proteins have also been devised and applied [Osborne, Mendel and Ferry (1919) and McCollum and co-workers (1921)]. These methods, however, accept as criteria either maintenance of nitrogen equilibrium or maintenance or increase in bodyweight. Several objections, such as differences in food intake, duration of experiment, and the omission of an allowance for the maintenance quota, have been raised against the latter methods, and it has been pointed out that the significance of results obtained under these conditions is in many instances difficult to assess. In their reviews on the biological values of proteins, Mitchell and Hamilton (1929), as well as Boas Fixsen (1935), have scrutinized and fully discussed these objections.

It appears from an analysis of the methods concerned with the determination of the biological value of proteins, that the balance-sheet method is the only one which discriminates clearly between absorbed and ingested nitrogen and consequently is not influenced in any way by differences in digestibility of the proteins. The biological value obtained by this method is a measure of the maximum percentage of absorbed nitrogen utilized in anabolism and can therefore, not be vitiated by differences in food intake as is the case where increase in weight is the sole criterion. In fact, the

general tendency is to regard the biological value of each protein or each protein mixture at a definite level of intake as a constant. This assumption is based on the generally accepted theory that the body's demand for tissue synthesis involves a rather constant proportion of the different amino acids. However, different proteins may differ appreciably in their respective biological values. This fact has been amply demonstrated since the time Rubner (1897) formulated the hypothesis that all proteins were not of the same value in nutrition. This difference in biological value does not only exist between different proteins, but also in the same protein depending on the level it is fed and the purpose it is destined for in the body.

The difference in biological value amongst different proteins is directly attributable to the varied amino acid constitution of the respective protein molecules, the difference at different levels of feeding being presumably due to the excess of amino acids being drawn into the oxidative processes of the body and catabolized, while biological differences of the same protein for different functions may be determined by the needs of the body for simple nitrogenous compounds on the one hand (maintenance) and a more complete assortment of amino acids on the other hand (growth).

It seems reasonable to explain the biological differences between proteins on the basis that proteins differ chemically in their proportions and amounts of indispensable amino acids. Some proteins may lack certain of these amino acids while others may be deficient in some other or a different combination of indispensable amino acids. A deficiency of one of these amino acids may limit partially or totally the rest of the amino acids in the protein molecule for growth. When two or more of these indispensable amino acids are lacking the utilization of the rest amino acids may be further impaired. Mitchell and Smuts (1932) have shown that when maize, which is deficient in both lysine and tryptophane is supplemented with lysine only, a small improvement in growth is obtained. When Tryptophane alone is fed in conjunction with maize no improvement in growth was noticed. If, however, both lysine and tryptophane are added to a maize ration significant increase in weight resulted. The more nearly, therefore, a protein approaches in composition the average amino acid mixture for the purpose it is intended for, the higher is its biological value. A perfect protein would be one which when ingested in amounts equal to or less than the output of nitrogen on a nitrogen free diet occasions no rise in the nitrogen output, thus showing that none of the amino acids present in the protein has been wasted.

It seems very improbable that the biological value of proteins for maintenance would be the same as that for growth or milk production. According to Mitchell (1929), whose theory appears to coincide best with the known facts, the minimum endogenous nitrogen (Maintenance) does not involve the disintegration of proteins, but is related entirely to the metabolism of non-protein nitrogenous constituents of the tissues. If such is the case it can readily be appreciated that incomplete mixtures of amino acids or incomplete proteins or even ammonium salts can be partially utilized to cover

the maintenance requirement of nitrogen, but can obviously not be utilized for growth. Hence it is apparent that some proteins may exhibit higher biological values for maintenance than for gorwth, since amino acid deficiencies may not limit protein utilization for maintenance, but certainly does for growth. In the latter case a deficiency of one of the indispensable amino acids may block synthesis of new tissues, with the result that diaminization of the amino acids takes place and a lowered biological value is obtained.

It is, therefore, evident that representative values of protein or mixture of proteins can be obtained only when the biological value is determined for each at different levels of intake and for the different functions in the body. However, it appears fairly hopeless to separate these functions to such an extent that the biological value of the proteins used can be assessed separately, since the physiological functions, such as growth and milk production, are superimposed on maintenance. Consequently the residual amino acids, which are not used for maintaining the integrity of the tissues, such as possibly lysine and cystine may enhance the biological value for growth. Therefore it becomes necessary to relate the nitrogen retained for both maintenance and growth to the nitrogen absorbed. In this publication, biological values give a composite measure of both maintenance and growth.

The biological determinations reported in this paper are based exclusively on the nitrogen balance sheet method and consequently reference will be made only to work carried out on the same basis. Nevens (1921) working with rats obtained a biological value of 62 for lucernemeal at approximately 11 per cent. level, while Satola (1930) on lambs found a value of 56. Mitchell (1936) in comparing biological values of raw and roasted peanuts found that raw peanut was slightly superior and showed a biological value of 57·8 at an 8 per cent. level in rats in comparison to 55·7 for the roasted product. Piang (1930) obtained a value for peanut closely approximating that obtained by Mitchell, namely 59·0. However, Holdaway, Ellett and Harris (1925) assigned values of 84, 78 and 77 to peanutmeal, cottonseedmeal and soyabeanmeal respectively for lactation. On the other hand, Morris and Wright (1933) for the same function obtained a biological value of peanutmeal of 50. In the latter case peanutmeal was supplemented by appreciable amounts of oats. Hence the biological value reported for peanutmeal is actually a supplemented biological value of peanutmeal plus oats.

Nevens (1921) in his detailed study with rats on the nutritive value of cottonseedmeal obtained a biological value of 66 at a level of approximately 11 per cent. Mitchell and Hamilton (1931) in a comparison of linseedmeal and cottonseedmeal in pigs at a 9 per cent. level found the biological value of the latter to be 63. On the other hand Braman (1931) from Mitchell's laboratory reported a value of 78 for cottonseedmeal at an 8 per cent. level of feeding. In a determination of the biological values of proteins at different levels Mitchell (1924) obtained values of 79 and 65 respectively for oats at a 5 and 10 per cent. level.

EXPERIMENTAL.

Rats were used as experimental animals. All determinations were carried out according to the method of Mitchell except that only one biological value was determined on a series of six rats. The nitrogen low period was conducted either prior to or after the protein period. In several cases nitrogen low periods were run before as well as after the protein period. The data, however, did not show any significant variation and consequently it was decided to run nitrogen low periods either before or after the period of protein feeding. Six-day periods were allowed on a nitrogen low ration for rats to adjust themselves to a constant nitrogen excretion. Rats were kept for the same length of time on the protein ration before collection started. Collection periods were of seven days' duration. Urine was collected in acid, and the daily faeces digested by the usual method. The week's digests of faeces were made up to known volume and aliquots distilled for nitrogen determination. The urine collected over the period was made up to a known volume and aliquots analysed for nitrogen, correction being made in the end volume for daily addition of acid. Rations were made up so as to contain approximately 9 per cent. of protein. Analyses, in duplicate, were made of all rations after mixing and before placing them in the icebox. In all cases the nitrogen low rations were equalized as far as possible in fibre content to the protein ration. The protein feeds tested were of the same nature as that fed under practical conditions except oats, which was a prepared product put out by a commercial company as a breakfast food.

RESULTS.

The nitrogen metabolism data as well as the calculation of the biological values are given in Table 2. The standardizing periods on the Nitrogen low ration preceded the protein feeding period in all cases except in the case of coprameal where the nitrogen low period was conducted after the protein period. The purpose of the nitrogen low periods is to determine the excretion of metabolic nitrogen in faeces per gram food consumed and of the endogenous urinary nitrogen per 100 grams bodyweight. These figures are assumed to be the same under protein administration and are, therefore, applied in the calculation of biological values as factors for estimating the contribution of the body of the nitrogen appearing in the faeces and the urine.

The accepted constancy of the endogenous nitrogen is naturally an important factor in the determination of biological values. The work of Smuts (1935) has provided additional evidence in confirmation of the constancy of the endogenous nitrogen by showing that a close correlation exists between the basal heat production and the endogenous nitrogen not only within species but among different species varying greatly in size. This finding would indicate that the constancy of the endogenous nitrogen is as well established as the basal heat production. Consequently any increase or decrease of the endogenous nitrogen must be regarded as an effect of either internal or external factors, which would influence the basal metabolism in a similar manner.

With regard to the metabolic faecal nitrogen, Schneider (1935) has shown that a portion is of body origin and that this portion may make up a considerable fraction of the total metabolic faecal nitrogen below maintenance. However, for levels of food intake usually employed in the determination of biological values, this fraction becomes insignificant in comparison with that related to the dry matter consumed. Consequently no correction for the portion related to the bodyweight was attempted and the entire metabolic faecal nitrogen was related to food consumed.

The biological value of lucernemeal was determined in two cases. Although there existed a difference of ·45 per cent. nitrogen between the two determinations, the biological values do not differ significantly. In the first instance the individual biological values varied from 55 to 66 with an average value of $60 \pm 1 \cdot 09$ and a coefficient of variation of 6 per cent., while in the second case the variation between individual values was from 60 to 63 with an average value of $61 \pm \cdot 463$ and a coefficient of variation of 2·3 per cent. There is a slight tendency for a higher biological value at the lower level of nitrogen feeding, but this difference is by no means significant. These values agree fairly well with the value of 62 obtained for rats by Nevens at a 10 per cent. level. The value of 56 for lucernemeal obtained by Satola for lambs at approximately the same level is in close agreement with the values obtained on rats.

No significant difference is manifested in the biological values of Peanutmeal, Sesamemeal and Coprameal, the values being $72 \pm 1 \cdot 19$, $71 \pm 1 \cdot 03$ and $69 \pm 1 \cdot 15$ respectively, with coefficients of variation of 5·5 per cent., 4·8 per cent. and 5·5 per cent. The absence of differences between the biological values of these proteins agrees very well with the results obtained from growth studies conducted on these proteins in an earlier paper by Smuts (1937). Mitchell's biological value of 57 and Piang's value of 59 for peanutmeal at approximately 8 per cent. level is much lower than the value reported in this paper. Holdaway Ellett and Harris, however, obtained a biological value of 84 for peanutmeal for milk production.

The biological values of oats and cottonseedmeal are distinctly superior to that of peanutmeal, copra and sesame meal, but do not show any difference between themselves. The biological value of oats is $84 \pm \cdot 79$ with a coefficient of variations of 1·9 per cent. and that of cottonseedmeal $81 \pm \cdot 93$ with a variability of 2·6 per cent. The latter value agrees well with the value of 79 obtained for cottonseedmeal by Braman at an 8 per cent. level. According to the biological value obtained by Mitchell of 79 for oats at 5 per cent. level, the value reported here is higher. Since this oat product is precooked as a breakfast food, it may easily account for the higher biological value. Hayward and Steenbock (1936) found similar results with heat-treated Soyabean in comparison with the raw product.

It would appear from the respective biological values that the proteins of oatmeal and cottonseedmeal are superior to that of sesamemeal, peanutmeal and coprameal and that the latter are again

superior to that of lucernemeal. However, such differences can best be established statistically. By analysing the variance of the entire data it is found that the variance between groups is significantly greater than the variation within groups, hence there exists a real difference between groups. The standard deviation as calculated from the variance within groups and the resulting coefficients of variation are found to be S.D. $= 3\cdot070$ and C of V $= 4\cdot32$ per cent. This indicates that the variability of individual determinations within groups is extremely small and compares favourably with the variability obtained in other biological determinations (Basal metabolism). The standard error of a mean of 6 biological values is given by $3\cdot07 \div \sqrt{6}$, i.e. $1\cdot25$, and this would allow the prediction of successive average biological values, with approximately 5 per cent. probability, to fall in the range of $\pm 2\cdot50$.

When the group means are considered it is found that significance between two means can be indicated by the following differences and probability. If a difference between means of 5 and 6 determinations respectively is greater than $5\cdot07$ the probability is less than 1 per cent. On the other hand, if the difference between two means of 6 determinations each is greater than $3\cdot60$ the probability of the difference is less than 5 per cent., but when the difference is $4\cdot83$ or exceeds it the probability of the difference becomes 1 per cent. or less. On the basis of these figures Table 3 has been constructed and compares the mean differences of the biological values of the different proteins. Black type indicate a highly significant difference while the ordinary type signifies no statistical differences of the average biological values.

From this table it is clear that the proteins of oatmeal and cottonseedmeal are superior to the rest of the proteins tested but do not differ significantly between themselves. Peanutmeal, coprameal and sesamemeal must, statistically speaking, be regarded as being utilized to the same extent in metabolism and significantly better than lucernemeal.

From Table 4 it will be seen that there is very little difference between the apparent and true digestibility of peanutmeal, sesamemeal, coprameal and cottonseedmeal. On this basis it must be assumed that the rat was able to utilize more of the absorbed nitrogen derived from cottonseedmeal than from the other three sources of protein, since it has a higher biological value. The apparent digestibility of oat protein is only $78\cdot8$ per cent. while if the body's contribution of faecal nitrogen be deducted the digestibility becomes 100 per cent., which would indicate that the protein of this particular oats is fully digested. The apparent and true digestibility of lucernemeal is much lower and would therefore partially account for its lower nutritive value in comparison with the other proteins.

Conclusions.

1. By means of nitrogen metabolism studies on rats the biological values were determined for oatmeal, cottonseedmeal, peanutmeal, sesamemeal, coprameal and lucernemeal. The values obtained for the respective proteins were 84, 81, 72, 71, 69, 60 and 61.

2. It is concluded on the basis of statistical analysis that the proteins of oatmeal and cottonseedmeal are equivalent in nutritive value for growth and maintenance, but superior to that of peanut, sesamemeal and coprameal. No difference was found between the proteins of peanut, sesamemeal and coprameal, which again are significantly superior to the proteins of lucernemeal.

3. The true digestibility of peanutmeal, sesamemeal, coprameal and cottonseedmeal was of about the same order, being 90, 92, 89 and 92 per cent., while that of lucernemeal was distinctly lower, namely 74 per cent. The protein of oatmeal appears to be entirely digested.

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TABLE 1.

Composition of Rations.

Ingredients.	A.	B.	C.	D.	E.	F.	G.
Peanut Meal.........	15·0	—	—	—	—	—	—
Lucerne Meal.........	—	57·5	—	—	—	—	—
Sesame Meal.........	—	—	25·0	—	—	—	—
Copra Meal...........	—	—	—	33·0	—	—	—
Cotton-seed Meal.....	—	—	—	—	22·5	—	—
Oatmeal.............	—	—	—	—	—	72·7	—
Sucrose..............	10·0	10·0	10·0	10·0	10·0	6·8	10·0
Butterfat............	8·0	8·0	8·0	8·0	8·0	6·0	8·0
Yeast Extract (1).....	10·0	10·0	10·0	10·0	10·0	8·0	10·0
Ether Ext. Egg White	—	—	—	—	—	—	3·8
Agar (2).............	—	—	—	—	—	—	2·0
Codliver Oil..........	2·0	2·0	2·0	2·0	2·0	2·0	2·0
NaCl................	1·0	1·0	1·0	1·0	1·0	—	1·0
Salt Mixture (3).......	4·5	4·5	4·5	4·5	4·5	4·5	4·5
Starch...............	49·5	7·0	39·5	31·5	42·0	—	58·7
Percent Nitrogen...	1·36	1·55	1·51	1·51	1·50	1·42	·654

(1) Yeast extract was prepared according to the method of (Itters, Orent E.R. and McCollum E. V. (J.B.C. 108–2–571–577–1935].

(2) The agar was raised to 14 per cent. in the nitrogen low ration for the determination of the biological value of lucerne meal.

(3) A modified Osborne and Mendel salt mixture prepared by P. B. Hawk and B. L. Oser (1931 Science Vol. 74, p. [illegible]

TABLE 2.

Nitrogen Metabolism Data and the Calculations of the Biological Values of Different Proteins.

Animal No.	Initial Weight gm.	Final Weight gm.	Average Weight gm.	Daily Food Intake. gm.	Daily N. Intake. mgm.	Daily Fecal N. mgm.	Body N. in Feces. Per Gram. Food. mgm.	Body N. in Feces. Per Day. mgm.	Food N in. Feces. mgm.	Absorbed N. mgm.	Daily Urinary N. mgm.	Body N. in Urine. Per 100 gms. mgm.	Body N. in Urine. Per Day. mgm.	Food N. in Urine. mgm.	Food N. Retained. mgm.	Biological Value.
N-Low Period for Lucerne Ration Containing ·654 Per cent. Nitrogen.																
19........	63·0	67·0	65·0	5·7	—	18·31	3·21	—	—	—	14·98	23·05	—	—	—	—
20........	82·0	88·0	85·0	8·0	—	21·60	2·51	—	—	—	17·28	20·33	—	—	—	—
21........	73·0	73·0	73·0	6·4	—	19·13	3·00	—	—	—	16·70	22·88	—	—	—	—
22........	76·0	78·0	77·0	6·3	—	20·37	3·23	—	—	—	18·46	24·00	—	—	—	—
23........	70·0	64·0	66·5	5·4	—	18·93	3·50	—	—	—	16·70	25·11	—	—	—	—
24........	64·0	63·0	64·0	5·6	—	18·12	3·24	—	—	—	17·28	27·00	—	—	—	—
Lucerne Ration Containing 1·55 Per Cent. Nitrogen.																
19........	74·0	82·0	78·0	8·6	133·3	58·40	3·21	27·61	30·79	102·51	61·32	23·05	17·98	43·34	59·17	58
20........	94·0	97·0	95·5	9·3	144·2	60·50	2·57	23·90	36·60	107·60	63·67	20·33	19·42	44·25	63·35	59
21........	84·0	91·0	87·5	9·3	144·2	56·79	3·00	27·90	28·89	115·31	65·48	22·88	19·92	45·56	69·75	60
22........	86·0	91·0	88·5	9·3	144·2	67·80	3·23	30·04	37·76	106·44	57·53	24·00	21·24	36·28	70·16	66
23........	74·0	82·0	78·0	8·0	124·0	65·70	3·50	28·00	37·70	86·30	25·11	25·11	19·59	38·81	47·49	55
24........	73·0	80·0	75·0	9·3	144·2	70·12	3·24	30·13	39·99	104·21	60·73	27·00	20·25	40·48	63·73	61
															Average......	60
N-Low Period for Peanut Ration Containing ·654 Per Cent. N.																
1........	45·0	46·0	45·5	3·7	—	8·20	2·19	—	—	—	17·80	28·13	—	—	—	—
2........	55·0	55·0	55·0	5·1	—	8·70	1·70	—	—	—	16·80	30·54	—	—	—	—
3........	49·0	48·0	48·5	4·0	—	7·20	1·82	—	—	—	11·87	24·60	—	—	—	—
4........	52·0	51·0	51·5	3·75	—	7·20	1·92	—	—	—	12·30	23·88	—	—	—	—
5........	48·0	48·0	48·0	3·3	—	8·26	2·18	—	—	—	10·91	23·70	—	—	—	—
6........	39·0	39·0	39·0	3·4	—	9·90	2·91	—	—	—	14·84	38·05	—	—	—	—
Peanut Ration Containing 1·36 Per Cent. Nitrogen.																
1........	47·0	50·0	48·5	3·4	46·24	10·97	2·19	7·45	3·52	43·72	27·81	28·13	13·64	14·49	29·23	67
2........	56·0	58·0	57·0	3·7	50·32	13·30	1·70	6·29	7·01	43·31	29·70	30·54	17·41	13·13	30·18	70
3........	50·0	53·0	51·5	4·3	58·48	14·90	1·82	7·83	7·07	51·41	29·52	24·60	12·67	11·93	39·48	77
4........	53·0	57·0	55·0	3·5	46·80	11·41	1·92	6·72	4·69	42·11	29·41	23·88	13·13	10·75	31·36	74
5........	48·0	50·0	49·0	2·9	39·44	7·82	2·18	6·32	1·50	37·94	23·20	23·70	11·61	12·09	25·85	68
6........	42·0	46·0	44·0	3·2	43·52	13·70	2·91	7·01	6·69	36·83	30·80	38·05	16·74	14·06	22·77	74
															Average......	72

TABLE 2—(*continued*).

Animal No.	Initial Weight gm.	Final Weight gm.	Average Weight gm.	Daily Food Intake. gm.	Daily N. Intake. mgm.	Daily Fecal N. mgm.	Body N. in Feces. Per Gram. Food. mgm.	Body N. in Feces. Per Day. mgm.	Food N in. Feces. mgm.	Ab-sorbed N. mgm.	Daily Urinary N. mgm.	Body N. in Urine. Per 100 gms. mgm.	Body N. in Urine. Per Day. mgm.	Food N. in Urine. mgm.	Food N. Re-tained. mgm.	Bio-logical Value.
N-LOW PERIOD FOR LUCERNE RATION CONTAINING ·654 PER CENT. N.																
8........	52·0	52·0	52·0	4·2	—	9·81	2·09	—	—	—	12·60	24·35	—	—	—	—
9........	52·0	52·0	52·0	4·7	—	10·00	2·13	—	—	—	11·50	22·12	—	—	—	—
10........	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11........	50·0	50·0	50·0	4·9	—	8·42	1·72	—	—	—	12·60	24·00	—	—	—	—
12........	50·0	50·0	50·0	4·6	—	10·30	2·24	—	—	—	10·90	21·80	—	—	—	—
13........	50·0	52·0	50·0	5·6	—	10·42	1·86	—	—	—	7·80	15·30	—	—	—	—
LUCERNE RATION CONTAINING 1·10 PER CENT. NITROGEN.																
8........	52·0	53·0	52·5	5·1	56·10	26·67	2·09	10·66	16·01	40·09	27·93	24·25	12·53	15·40	24·69	60
9........	53·0	52·0	52·5	4·9	53·90	26·04	2·13	10·44	15·60	38·30	26·31	22·12	11·61	14·70	23·60	62
10........	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11........	50·0	55·0	52·5	5·7	62·70	26·04	1·72	9·80	16·24	46·46	30·58	24·00	12·60	17·98	28·48	61
12........	49·0	53·0	51·0	8·0	55·00	23·31	2·24	11·20	12·11	42·89	28·11	21·80	11·12	16·99	25·90	60
13........	51·0	55·0	53·0	6·4	70·40	27·93	1·86	11·90	16·03	54·37	28·11	15·30	8·11	20·00	34·37	63
														Average......		61
N-LOW PERIOD FOR OATMEAL RATION CONTAINING ·654 PERCENT. N.																
42........	134·0	134·0	134·0	—	—	29·48	3·68	—	—	—	20·90	15·60	—	—	—	—
43........	111·0	105·0	108·0	—	—	26·70	4·05	—	—	—	22·00	20·37	—	—	—	—
44........	109·0	105·0	107·0	—	—	20·68	3·28	—	—	—	28·60	26·73	—	—	—	—
45........	120·0	115·0	117·5	—	—	16·94	2·82	—	—	—	27·72	23·59	—	—	—	—
46........	109·0	105·0	107·0	—	—	19·80	3·30	—	—	—	20·46	18·79	—	—	—	—
47........	127·0	125·0	126·0	—	—	33·44	4·18	—	—	—	23·76	18·86	—	—	—	—
OATMEAL RATION CONTAINING 1·42 PER CENT. NITROGEN.																
42........	137·0	144·0	140·5	8·0	113·60	26·18	3·68	29·44	0·0	113·60	39·01	15·60	21·92	17·09	96·51	85
43........	115·0	122·0	118·5	8·0	113·60	23·61	4·05	32·40	0·0	113·60	44·39	20·37	24·14	20·25	93·35	82
44........	120·0	124·0	122·0	8·0	113·60	24·33	3·28	26·24	0·0	113·60	50·30	26·73	32·61	17·69	95·91	84
45........	125·0	135·0	130·0	8·0	113·60	22·59	2·82	22·56	0·03	113·57	47·23	23·59	30·67	16·56	97·01	85
46........	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
47........	135·0	137·0	136·0	8·0	113·60	25·67	4·18	33·44	0·0	113·60	41·10	18·86	25·65	15·45	98·15	86
														Average......		84

TABLE 2—(*continued*).

Animal No.	Initial Weight gm.	Final Weight gm.	Average Weight gm.	Daily Food Intake. gm.	Daily N. Intake. mgm.	Daily Fecal N. mgm.	Body N. in Feces. Per Gram. Food. mgm.	Body N. in Feces. Per Day. mgm.	Food N in. Feces. mgm.	Ab-sorbed N. mgm.	Daily Urinary N. mgm.	Body N. in Urine. Per 100 gms. mgm.	Body N. in Urine. Per Day. mgm.	Food N. in Urine. mgm.	Food N. Re-tained. mgm.	Bio-logical Value.
						N-LOW PERIOD FOR SESAME MEAL RATION CONTAINING ·654 PER CENT. NITROGEN.										
101........	71·0	78·0	74·5	7·2	—	19·15	2·65	—	—	—	18·35	24·63	—	—	—	—
102........	71·0	76·0	73·5	7·2	—	15·12	2·10	—	—	—	15·60	21·22	—	—	—	—
103........	77·0	74·0	75·5	6·7	—	18·39	2·74	—	—	—	16·10	21·32	—	—	—	—
104........	70·0	77·0	73·5	7·2	—	18·00	2·50	—	—	—	18·62	25·33	—	—	—	—
105........	65·0	68·0	66·5	6·7	—	14·36	2·14	—	—	—	14·34	21·56	—	—	—	—
106........	75·0	79·0	77·0	7·2	—	19·15	2·64	—	—	—	17·61	23·00	—	—	—	—
						SESAME MEAL RATION CONTAINING 1·51 PER CENT. NITROGEN.										
101........	82·0	97·0	89·5	9·6	144·96	30·20	2·65	25·44	4·76	140·20	55·87	24·63	22·04	33·83	106·37	76
102........	77·0	85·0	81·0	8·4	126·84	30·20	2·10	17·64	12·56	114·28	53·93	21·22	17·19	36·74	77·54	68
103........	77·0	84·0	80·5	7·4	111·74	28·69	2·74	20·28	8·41	103·33	47·67	21·32	17·16	30·51	72·82	70
104........	78·0	96·0	87·0	8·8	132·88	31·28	2·50	22·00	9·28	123·60	54·80	25·33	22·03	32·77	90·83	73
105........	75·0	85·0	80·0	7·6	114·76	31·93	2·14	16·26	15·67	99·09	48·32	21·56	17·25	31·07	68·02	69
106........	83·0	95·0	89·0	8·0	120·80	31·49	2·64	21·12	10·37	110·43	56·92	23·00	20·47	36·45	73·98	67
															Average......	71
						N-LOW PERIOD COTTON SEED MEAL CONTAINING ·654 PER CENT. N.										
1........	92·0	95·0	93·5	7·0	—	19·86	2·84	—	—	—	—	18·87	—	—	—	—
2........	82·0	92·0	87·0	7·0	—	14·98	2·14	—	—	—	—	26·10	—	—	—	—
3........	97·0	94·0	95·0	6·4	—	19·34	3·02	—	—	—	—	20·23	—	—	—	—
4........	97·0	97·0	97·0	7·0	—	22·96	3·28	—	—	—	—	20·30	—	—	—	—
5........	82·0	80·0	81·0	6·0	—	16·92	2·82	—	—	—	—	20·74	—	—	—	—
6........	102·0	102·0	102·0	6·6	—	18·74	2·84	—	—	—	—	21·14	—	—	—	—
						COTTON SEED RATION CONTAINING 1·50 PER CENT. NITROGEN.										
1........	95·0	104·0	99·5	8·1	121·50	34·08	2·84	23·00	11·08	110·42	32·14	18·87	18·68	24·46	85·96	78
2........	87·0	100·0	93·5	8·1	121·50	34·29	2·14	17·33	16·96	104·54	45·81	26·10	24·40	21·41	83·13	80
3........	87·0	95·0	91·0	8·0	120·00	34·08	3·02	24·16	9·92	110·08	36·24	20·23	18·41	17·83	92·25	84
4........	95·0	107·0	101·0	8·1	121·50	32·36	3·28	26·57	5·79	115·71	43·14	20·30	20·50	22·64	93·07	80
5........	80·0	92·0	86·0	8·0	120·00	31·93	2·82	22·56	9·37	110·63	39·26	29·74	17·84	21·42	89·21	81
6........	95·0	105·0	100·0	8·0	120·00	29·34	2·84	22·72	6·62	113·38	41·42	21·14	21·14	20·28	93·10	82
															Average......	81

TABLE 2—(*continued*).

Animal No.	Initial Weight gm.	Final Weight gm.	Average Weight gm.	Daily Food Intake. gm.	Daily N. Intake. mgm.	Daily Fecal N. mgm.	Body N. in Feces. Per Gram. Food. mgm.	Body N. in Feces. Per Day. mgm.	Food N in. Feces. mgm.	Ab-sorbed N. mgm.	Daily Urinary N. mgm.	Body N. in Urine. Per 100 gms. mgm.	Body N. in Urine. Per Day. mgm.	Food N. in Urine. mgm.	Food N. Re-tained. mgm.	Bio-logical Value.
N-LOW PERIOD COPRA MEAL CONTAINING ·654 PER CENT. N.																
7........	92·0	90·0	91·0	7·4	—	26·59	3·59	—	—	—	16·91	18·58	—	—	—	—
8........	82·0	87·0	84·5	7·4	—	19·93	2·70	—	—	—	17·38	20·57	—	—	—	—
9........	78·0	80·0	79·0	7·4	—	17·52	2·37	—	—	—	18·37	23·24	—	—	—	—
10........	88·0	92·0	90·0	7·4	—	23·56	3·18	—	—	—	19·81	22·00	—	—	—	—
11........	81·0	87·0	84·0	7·4	—	18·12	2·45	—	—	—	20·77	24·73	—	—	—	—
12........	86·0	94·0	90·0	7·4	—	17·52	2·37	—	—	—	14·49	16·10	—	—	—	—
COPRA MEAL RATION CONTAINING 1·51 PER CENT. NITROGEN.																
7........	87·0	94·0	90·5	7·0	105·70	35·16	3·59	25·13	10·03	95·67	47·61	18·58	16·81	30·80	64·87	68
8........	79·0	90·0	84·5	8·1	122·31	30·41	2·70	21·87	9·57	112·74	49·35	20·57	17·48	31·87	80·87	72
9........	74·0	82·0	78·0	7·4	111·74	33·22	2·37	17·54	15·68	96·06	46·60	23·24	18·13	28·47	67·57	70
10........	85·0	97·0	91·0	8·1	122·31	41·90	3·18	25·76	16·14	106·17	48·15	22·00	20·02	28·13	78·04	74
11........	76·0	87·0	81·5	8·0	120·80	33·22	2·45	19·60	13·62	107·18	60·00	24·73	20·14	39·86	67·32	63
12........	85·0	93·0	89·0	7·7	116·27	28·90	2·37	18·25	10 65	105·62	48·15	16·10	14·33	33·82	71·80	68
															Average......	69

Table 3.

Statistical Analysis of Difference between Means.

	Oatmeal.	Cotton Seed Meal.	Peanut Meal.	Sesame Meal.	Copra Meal.	Lucerne Meal.
Oatmeal................	—	3·57	**12·73**	**13·90**	**15·23**	**23·20**
Cotton Seed Meal........	—	—	**9·16**	**10·33**	**11·66**	**19·63**
Peanut Meal............				1·17	2·50	**10·44**
Sesame Meal............	—	—		—	1·35	**9·30**
Copra Meal........	—	—	—	—	—	**7·97**
Lucerne Meal					—	—

Table 4.

The Digestibility of the Nitrogen in Lucernemeal, Peanutmeal, Oatmeal, Sesamemeal, Cottonseedmeal and Coprameal.

Rat No.	Lucerne Meal. Apparent Digestibility.	Lucerne Meal. True Digestibility.	Rat No.	Peanut Meal. Apparent Digestibility.	Peanut Meal. True Digestibility.	Rat No.	Lucerne Meal. Apparent Digestibility.	Lucerne Meal. True Digestibility.	Rat No.	Oatmeal. Apparent Digestibility.	Oatmeal. True Digestibility.
19	55·3	76·9	1	76·3	94·6	8	52·5	71·5	42	77·0	100·0
20	58·0	74·6	2	73·6	86·1	9	5·17	71·1	43	79·2	100·0
21	60·6	79·7	3	74·5	88·0	10	—	-	44	80·3	100·0
22	66·9	73·8	4	75·6	90·0	11	58·5	74·1	45	80·1	100·0
23	47·8	69·6	5	80·2	96·2	12	61·3	78·0	46	—	—
24	51·4	72·3	6	68·5	84·6	13	60·3	77·2	47	77·4	100·0
Ave.	56·7	74·5		74·8	89·9		56·9	74·4		78·8	100·0

Rat No.	Sesame Meal. Apparent Digestibility.	Sesame Meal. True Digestibility.	Rat No.	Cotton Seed Meal. Apparent Digestibility.	Cotton Seed Meal. True Digestibility.	Rat No.	Copra Meal. Apparent Digestibility.	Copra Meal. True Digestibility.
101	79·2	96·7	1	72·0	90·9	7	66·7	90·5
102	76·2	90·1	2	71·8	86·0	8	75·1	92·2
103	74·3	92·5	3	71·6	91·7	9	70·3	86·0
104	76·5	93·0	4	73·3	95·2	10	65·7	86·8
105	72·2	86·3	5	73·3	92·2	11	72·5	88·7
106	82·2	91·4	6	75·6	94·4	12	75·1	90·8
Average	76·8	91·7		72·9	91·7		70·9	89·2

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Sulphur Metabolism.

V.—The Effect of Elementary Sulphur on Fertility, Reproduction and Lactation in the White Rat.

By J. H. KELLERMANN, Section of Biochemisty and Nutrition, Onderstepoort.

WHEN a drug is administered over an extended period of time not only its prophylactic effect but also its influence on reproduction should be considered. Some apparently harmless compounds may, under certain conditions, have disastrous effects on the organism, and the discovery by Waddell and Steenbock (1928) of the destruction of vitamin E in foodstuffs by ferric chloride illustrates again the necessity for collecting information on the effects of compounds in various combinations before they are used in practical applications. Therefore, the object of this experiment is to throw light on the effects of sulphur on reproduction and lactation.

EXPERIMENTAL.

The first generation of animals in this experiment consisted of 48 young rats—24 males and 24 females. They were equally divided into two groups with respect to sex, litter and weight. The rats were started on experiment when they were exactly 28 days of age and kept in individual cages on raised screen bottoms until they were 126 days of age. The screens were then removed, wood shavings added and a male put with every female. In all succeeding generations the rats were kept on wood shavings and weaned and mated when they were 28 and 126 days of age respectively.

The rats were weighed once weekly, fed *ad libitum* and allowed free access to distilled water. In order to study the effect of sulphur on food utilization and gain, which might help to elucidate the ultimate effect of sulphur on reproduction and lactation, an accurate record was kept of the food consumption for the first 14 weeks of the experiment. Both groups received an ordinary stock ration with the exception that three parts of elementary sulphur replaced an

equivalent amount of maize meal in the food of the one group. Furthermore the diets were made equicaloric by replacement of some of the maize meal with an isodynamic quantity of lard. The composition of the rations and the gains and efficiency quotients of the rats maintained on them are given in tables I and II respectively.

TABLE I.

Composition of Rations in Percentage by Weight.

	Basal Ration.	Sulphur Ration.
Yellow maize meal	68	62·6
Linseed Oil Meal	10	10
Crude casein	5	5
Brewers' Yeast	5	5
Lucerne Meal	3	3
Butter Fat	5	5
Beef Liver (dried at 70°C)	2	2
Bone Ash	1	1
$CaCO_3$	0·5	0·5
NaCl	0·5	0·5
Flowers of Sulphur	—	3·0
Lard	—	2·4

TABLE II.

Comparison of Gains and Efficiency Quotients of Rats fed Stock Diet with and without 3 per cent. Elementary Sulphur (equicaloric) for 14 weeks by ad libitum *method.*

Gain.		Mean Weight.		Total Food.		Efficiency Quotient.	
Range g.	Mean g.	Range g.	Mean g.	Range g.	Mean g.	Range	Mean
CONTROL GROUP. *Females.*							
118—174	141·3	128—175	151·7	1087—1484	1276	4·93—7·20	5·95
Males.							
217—326	272·5	198—284	238·0	1368—1814	1629	1·93—3·17	2·51
SULPHUR GROUP. *Females.*							
111—178	139·0	126—178	149·0	1031—1538	1205	4·45—8·20	5·82
Males.							
180—297	257·5	183—260	227·8	1305—1800	1549	2·25—3·96	2·64

The data in table II seem to show that the animals in the sulphur group consumed slightly less food and gained correspondingly less than their control mates. The weighted means for the males and females show food consumption of 1452 and 1377 g. and gains of 206·9 and 198·2 g. for the control and sulphur groups respectively. These differences, obtained over so long a period, are so small that they can be disregarded. Furthermore, it is clear, that the animals on the control and sulphur rations utilized their food to the same extent. The efficiency quotients, calculated according to the method of Palmer and Kennedy (1929)* were 5·95 and 5·82 for the females and 2·51 and 2·64 for the males of the respective control and sulphur groups. The marked difference obtained between the efficiency index of male and female rats substantiates the results of Palmer and Kennedy (1931).

However, a ration may support good growth yet fail to maintain the weight of lactating animals. That was the experience of Slonaker (1931) whose nursing females lost in weight on diets containing from 10 to 26 per cent. of proteins. In order, therefore, to ascertain whether the females maintained their weight while nursing on the diets used in this experiment, they were weighed at parturition and again after 19 days of lactation. The results are tabulated in table III.

TABLE III.

Comparison of gains of Female Rats during first 19 days of Lactation fed a Stock Diet with and without 3 per cent. of Elementary Sulphur.

Number of Lactating Females.	During First 19 Days of Lactation.					
	Number of Suckling Young.		Gain in Weight† by Mother.		Percentage Gain by Mother.	
	Range.	Average	Range g.	Average g.	Range.	Average
		Control Group. 1st Generation.				
10.............	4–10	6·5	3–32	19·7	1·1–13·7	8·55
		Sulphur Group. 1st Generation.				
9.............	4–9	6·2	9–58	25·2	3·2–28·3	11·4
		2nd Generation.				
16.............	4–9	6·5	2–38	19·7	1·0–18·4	9·6
		3rd Generation.				
11.............	2–9	6·2	10–40	20·2	5·0–19·8	10·
Average for S group 12...........	—	6·3	—	21·7.	—	10·3

* The dry matter only, not the digestible dry matter, was used in these calculations.

† Except for one female in 3rd generation of sulphur group, all the females gained in weight during the first 19 days of lactation. This female suffered from a lung infection and she and her offspring were therefore not used in the results.

TABLE IV.

Comparisons of Fertility and Reproduction of Rats fed a Stock Diet with and without 3 per cent. of Elementary Sulphur.

Average Size of Litter Born.	Average Percentage of Litter Weaned.	Average Weight of Males.		Average Weight of Females.		Weight Gained during First 28 Days.					
						Males.			Females.		
		At Birth. g.	At Weaning. g.	At Birth. g.	At Weaning. g.	Total g.	Average Daily g.	Gain. Per Cent.	Total g.	Average Daily g.	Gain. Per Cent.
					Control Group. *1st Generation.*						
8·3	78·31	4·78	55·72	4·47	50·32	50·94	1·819	38·05	45·85	1·637	36·62
					Sulphur Group. *1st Generation.*						
9·1	68·29	4·59	46·00	4·60	45·53	41 41	1·479	32·22	40·93	1·462	31·78
					2nd Generation.						
7·6	86·78	4·53	50·73	4·45	47·48	46·20	1·650	36·42	43·03	1·537	34·54
					3rd Generation.						
8·3	74·72	4·68	42·73	4·34	38·43	38·05	1·359	29·04	34·09	1·217	28·04
Grand Average for S. group. 8·3	76·59	4·60	46·49	4·46	43·81	41·89	1·496	32·52	39·35	1·405	31·50

It is evident that even after three generations the incorporation of 3 per cent. of elementary sulphur in the stock ration had no appreciable effect on the weight of the nursing mothers. The average number of young nursed for the first 19 days after parturition was 6·5 and 6·3 and the percentage gains in weight by the mothers were 8·55 and 10·3 for the control and sulphur groups respectively. Furthermore, in no case, but one, did the nursing mothers lose in weight. This does not substantiate the observations of Slonaker and the author therefore agrees with MaComber (1934) that Slonaker's diet was probably defective in certain respects.

The results given in table IV show that elementary sulphur had no effect on the fertility and reproduction in rats. The average size of the litters born was 8·3 and 8·3 and the percentages weaned 78·31 and 76·59 for the control and sulphur groups respectively.

Moreover, the weight of the young at birth did not differ appreciably when the two groups are compared. The average weights for the males were 4·78 and 4·60 g. and for the females 4·47 and 4·46 g. for the respective groups. However, the young from control mothers were slightly heavier at weaning than those from mothers fed the sulphur ration. The total gains were 50·94 and 41·89 g. for the males and 45·85 and 39·35 g. for the females for the control and sulphur groups, respectively, with corresponding differences in the average daily gains expressed in grams or percentages. Nevertheless these differences are so small that they seem to be insignificant.

While these experiments were in progress, Daggs (1935) published his technique for studying the lactation in rats. Consequently it was thought of interest also to apply his method in studying the effect of sulphur on lactation. The rats in the control group were from the second and those in the sulphur group from third generation rats fed the respective rations. They were weaned and mated at the ages stated previously. At parturition the litters were reduced to six except for those below this number which were discarded. The results are given in table V.

TABLE V.

Comparison of Lactation in Rats fed a Stock Diet with and without 3 per cent. of Elementary Sulphur.

Ration.	No. of Litters.	K_1.		K_2.		Lactation Index.	
		Range.	Average.	Range.	Average.	Range.	Average.
Control....	14	·0410–·0615	·0515	·0213–·0466	·0365	750–1000	880
Sulphur....	10	·0466–·0635	·0523	·0300–·0475	·0370	776–1110	893

The lactation indices were found to be 880 and 893 for the control and sulphur groups, respectively, from which it is evident that the feeding of sulphur had no effect on the lactation in rats.

SUMMARY.

1. Data are presented on the effect of incorporating 3 per cent. of elementary sulphur in a stock ration on the reproduction and lactation in rats.

2. Under the experimental conditions elementary sulphur had no effect on either fertility, reproduction or lactation in rats.

3. There was no difference between the birth weights of young from the control and sulphur groups. However, at weaning the offspring from control rats weighed slightly more than those from sulphur fed animals.

4. The extent to which the food was utilized by the sulphur group was similar to that of the control one. Furthermore, the lactating rats in both groups gained in weight during the first nineteen days after parturition.

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Section IV.

Sheep and Wool.

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The Extraction of Wool Grease.

By S. D. ROSSOUW, Section of Wool Research, Onderstepoort.

THE medical importance of wool grease was appreciated by the ancient Greeks as early as 450 B.C. Through mediaeval periods its use is referred to in various writings, and the methods of preparation are discussed. Galen prepared an unguent having wool grease as its basis and this formula has persisted with little change until modern times. Even today it finds a wide application in medicine and particularly veterinary medicine, in the form of lanoline. Many firms, especially in Germany, specialise in these products. Lately Twort and Twort (1934) have established the value of lanoline treatment in preventing dermatitis and cancer where employees are in contact with the highly carcinogenic oils used in the textile industry. The protective action of wool grease on the fibre is well known and wool buyers attach much value to its presence and even distribution. It is considered by some to be of nutritive value to the fibre during its growth.

There appears to be little uniformity as to the nomenclature for the product of the sebaceous gland. Many writers prefer the name "wool fat". This term is definitely misleading because the natural product contains very little true fat, i.e. glycerides. On the Continent "wool wax" is more often used. From the chemical point of view this is more correct. "Wool grease" is popularly used and, although chemically, it signifies little or nothing, it appears to be an acceptable name for this heterogeneous mixture of chemical compounds for the reason that "grease" does not specify any particular chemical combination.

It is problematic which compounds should be classed under "wool grease" and which under "suint". Of the potassium salts of the fatty acids, that combination which is principally present, those of the higher members of the series dissolve to an appreciable extent in ether and will, therefore, be found in the ether extract of raw wool. The lower members, on the other hand, are soluble only in water and contribute a great part of the so-called suint.

As the differentiation mostly centres round products of suderiforous and sebaceous excretion respectively the products should be grouped in this fashion as far as possible. The interaction of akali from the suderiforous glands with free fatty acid from the sebaceous glands may form water soluble fatty acid salts, thus introducing an element of confusion. By regenerating these fatty acids from their salts in the suint and adding these to the " grease ", the result may be regarded as fairly true for " grease ". This decision is made because the water extract can never be a true index of suderiforous excretion. The water extract will include the water soluble foreign material of sand, dust, vegetable matter and manure, thus including organic and inorganic matter from these sources.

The object of this paper is to describe an improved method of extraction of wool grease without reference to the chemical composition or analysis of wool grease and suint. It is definitely known, however, that wool grease is rich in sterols including such substances as cholesterol, the so-called iso-cholesterol ($C_{26}H_{44}O$) and smaller quantities of lanolesterol and agnosterol, as separated by Freney (1934). There is a deplorable lack of corroborative data as to the final constituents of wool grease, mainly due to the absence of suitable analytical methods. Herbig (1926) reviewed many methods but expressed strong criticism of most of them. The standard analytical methods for analysis of fats and waxes cannot be applied directly to wool grease because it contains so many chemical compounds, not usually found in fats or waxes.

As regards laboratory methods for extraction of wool grease, there is a conspicuous lack of uniformity resulting in products of differing composition. Benzol, petroleum ethers of various boiling points, and motor spirits have all been used at times but the solvent most frequently employed would appear to be ethyl ether. Sutton (1931) dried wool at 105° C. and used dry ether, basing results on the weight of dry wool. Marston (1928) employed dry ether apparently on unconditioned wool and extracted for 48 hours.

The procedure adopted for the present was as follows: 50 gm. of greasy wool from the right shoulder is conditioned to 70° F. (21·5° C.) and 70 per cent. relative humidity until constant weight is obtained. It was then placed in a fine texture Whatman extraction thimble and dried over sulphuric acid for at least 12 hours at 70° C. under a vacuum of 25 mm. It is then quickly weighed and placed in a soxhlet extractor protected by a calcium chloride tube attached to the condenser. This preliminary drying prevents coloidal matter from passing through the filter during the subsequent extraction.

In looking for a suitable solvent it was found that all those with the higher boiling points tended to produce a dark coloured grease. Redistilled petroleum ether of boiling point of approximately 45° C. was found to be most suitable. Drummond and Baker (1929) in their valuable work on wool fat used petroleum " spirit " of boiling point of approximately 60° C. Replacement by syphoning of this

45° C. petroleum ether five times seems to extract most of the grease, for continuation of the extraction for twenty runnings only produces a further addition of an average of 0·45 per cent. of the total grease, extreme figures being 0·32 per cent. and 0·65 per cent. of the total grease. As no solvent, except probably benzol, was found to extract all the grease, the petroleum ether extracted samples were again extracted with dry ether which delivered a further 0·75 per cent. of the total grease. This ether extract, which was now perfectly clear, seems to have a much higher acid value than the bulk, acid values of approximately 100 being found, whereas the bulk usually shows about 10. This indicates that some of the free acids (possibly oxy-acids) are practically insoluble in petroleum ether. It will be realised that for most grease determinations it will be satisfactory to employ a low boiling point petroleum ether and to pass this through about ten times. The extracts were evaporated at a temperature of 50° C. and the flasks dried out in the vacuum dessicator previously employed (25 mm. and 70° C.) until constant in weight (approximately 6 hours).

Suint was determined by soxhlet extracting the degreased wool with hot water, evaporating on the water bath, drying as above and weighing the residue as rapidly as possible. Should it be felt desirable, the fatty acids should be liberated by acidification, extracted with ether and this quantity added to the petroleum ether and ether extracts already obtained. The pure dry wool values were obtained by applying the method of Botha (1937), where a weighed quantity of the extracted wool is dissolved in boiling normal caustic soda. The residue is then retained on a fine mesh sieve, dried, weighed and a correction factor brought in due to loss of weight as a result of the alkali attacking the vegetable matter to some extent. This gives the weight of foreign material and by difference that of wool.

Grease figures are often expressed as a percentage of the raw wool conditioned at 70° F. and 70 per cent. relative humidity. A more correct method is that described by Sutton (1931) and later by Bonsma (1934), where use is made of a fat or grease index which, at the same time, is also a percentage expressed in terms of pure dry wool.

$$\text{Grease index} = \frac{\text{Weight of grease} \times 100.}{\text{Weight of pure dry wool.}}$$

The final result is thus not affected by variation in suint, vegetable matter, water and particularly sand content. The method based on raw wool is only of value where actual yield is required as is the case with wool buyers, whereas the figures obtained by the "grease index" can easily be converted to suit this purpose if necessary.

The following results were obtained from the wool of the sheep in the sulphur supplement experiment of Steyn (1935).

	No.	Grease.	Suint.	Pure Dry wool.
Sulphur Supplement..............	1	48·9	11·6	49·8
	2	41·9	23·9	49·9
	3	42·8	14·5	52·3
	4	52·6	14·0	54·1
	5	44·8	10·5	47·5
	6	67·1	20·5	52·1
	Average	49·7 ± 3·8	15·8 ± 2·1	51·0 ± 1·0
Controls......................	7	46·0	18·2	50·2
	8	43·0	10·1	54·1
	9	36·3	12·2	50·0
	10	28·7	18·4	46·1
	11	54·2	13·5	49·4
	12	42·3	16·2	44·1
	13	39·2	10·6	54·8
	14	40·9	13·4	53·1
	Average	41·3 ± 2·6	14·1 ± 1·1	50·2 ± 1·3

Although there appears to be a difference in the average grease content between the groups it is obvious that none of the differences could be considered statistically significant, indicating that the sebaceous and suderiforous excretions have not changed as a result of the sulphur supplement. The increase in weight which Steyn (1935) found is thus not due to the increase of wool grease or suint.

This method of extraction not only seems to produce consistent results but has the additional advantage of extracting the grease more fully and apparently also in a purer form. It is a comparatively quick method when extremely accurate results are not required.

Summary.

A method for the extraction of wool grease by means of low boiling point petroleum ether is described.

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Section V.

Miscellaneous.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 10, Number 1, January, 1938.

Printed in the Union of South Africa by the Government Printer, Pretoria.

Automatic Control of Fixation Time of Histological Specimens.

By CECIL JACKSON, Section of Anatomy, Onderstepoort.

An inexpensive and effective device to facilitate the process of fixing normal and pathological histological specimens in fluids which have a narrow margin of optimum fixation time has been in daily use at Onderstepoort for some years. It has proved of such great convenience that it appears advisable to bring it to general notice.

At this Institute a number of specimens for histological examination, especially those accruing from autopsies and biopsies, are fixed in Helly's Fluid (or one of the other modifications of Zenker's), which for many purposes is superior to formalin and for some purposes quite indispensable. The period of fixation varies from about four hours to about twelve hours, according to the nature of the specimen, so that a return to the laboratory after working hours would often be necessary in order to terminate the fixation, while specimens received in the afternoon require attention at night.

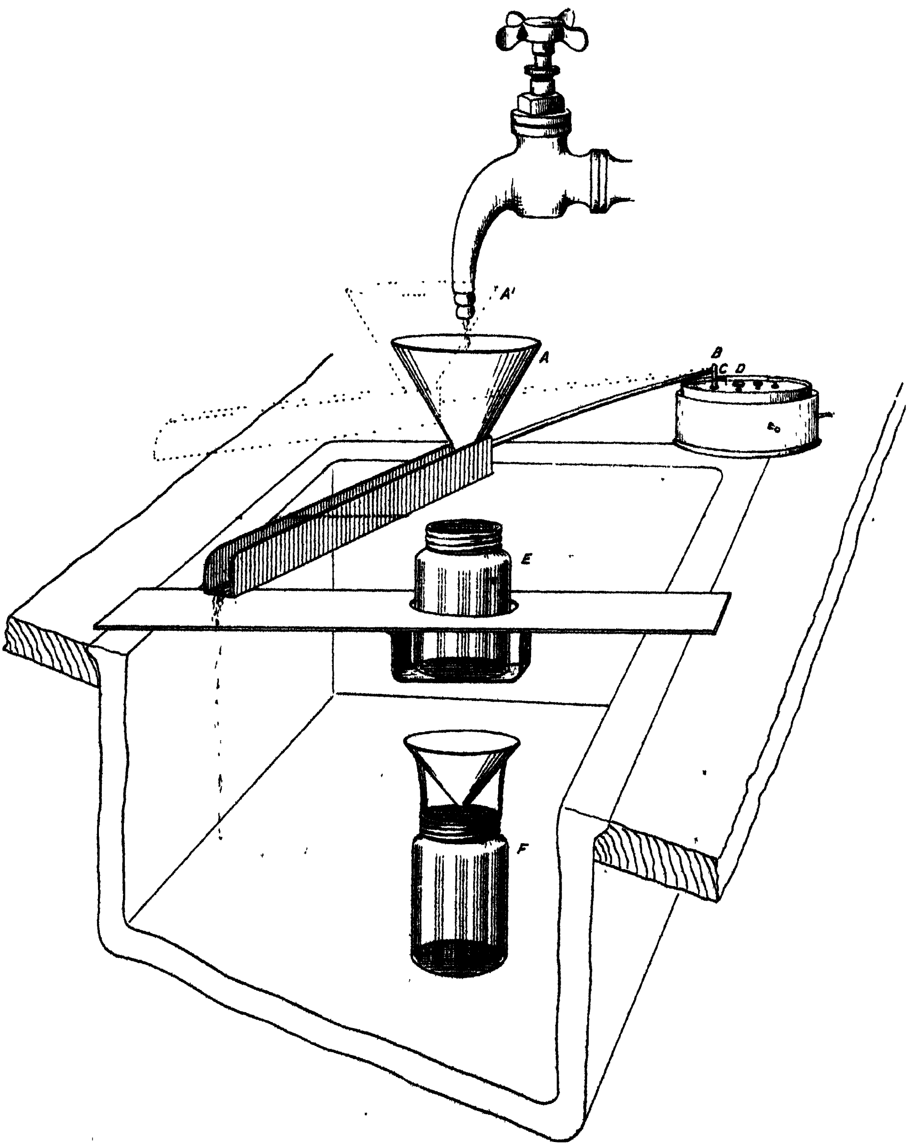
The apparatus (see illustration) comprises a household alarm clock of superior type, to the alarm-winder of which is soldered a metal spindle (B); this carries a detachable arm terminating in a "diverter" (A), which consists of a metal funnel and gutter. The tissues are immersed in the fixing fluid in a screw-top bottle (E), the central portion of the lid of which is replaced by a circle of wire-gauze. The bottle is supported by a well in a strip of metal, one end of which may be placed against the water pipe of the sink and the other against a mark on the opposite edge of the sink, ensuring that the bottle will be directly below the tap.

When fixation is begun, the alarm is set to the required time and the clock placed face downward at one side of the sink. The arm carrying the diverter is attached and adjusted to divert the water from the gently running tap. On the alarm being released, the diverter swings aside to a position (A1), which is limited by the stop (C) coming into contact with the alarm-setter (D), and the water runs into the bottle, displacing the fluid and thereafter washing the tissues.

A second bottle (F), fitted with a funnel to catch the overflow from the first, is of use when the amount of tissue is too large for one bottle or when specimens from different sources have to be kept separately.

The method is further of convenience at any time of the day, ensuring against forgetfulness in the termination of fixation, and is readily adapted to the staining of smears.

Acknowledgement is made to Mr. F. D. Horwell, of this Institute, for fitting up the apparatus and for valuable suggestions regarding the details of the design; and to Mr. C. G. Walker for the accompanying drawing.



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P. J. DU TOIT,
Director of Veterinary Services.

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Section I.

Bacteriology.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 10, Number 2, April, 1938.

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Variation in the Colony Form of the Anthrax Bacillus.

By MAX STERNE, Section of Bacteriology, Onderstepoort.

THE number of variant forms which can develop in cultures of the anthrax bacillus seems to be very large. (Gratia 1924; Nungester 1929; Stamatin 1934, 1935a, 1935b; Januschke 1935.) Some changes in colony form are so abrupt and occur so regularly that they appear, at first sight, to be stages of a life cycle or a life sequence. For example, all virulent strains investigated here (Sterne 1937a and 1937b) have produced a smooth mucoid growth on serum agar in an atmosphere containing certain concentrations of carbon dioxide; and all have, under such conditions, rapidly and continuously produced avirulent daughter strains. Nevertheless, the majority of variant colony types occur in a more haphazard manner so that it is usually impossible to predict, with any assurance, the changes that will occur in a culture subjected to certain conditions.

The results summarized below were obtained during the course of experiments done to see whether any "trends" could be recognized in the dissociation pattern of *B. anthracis*.

EXPERIMENTS.

Dissociation of Strain XXII.

This strain was grown on 50 per cent. horse serum agar in 20 per cent. carbon dioxide immediately after its isolation from an animal which had died of anthrax during a natural outbreak of the disease. From the resulting smooth mucoid culture a rough, avirulent, and uncapsulated variant was obtained. This was used in further experiments on dissociation because its inability to produce capsules considerably simplified observation and moreover, dissociation as far as it affects the capsule was discussed in a previous paper (Sterne 1937 a). See also Stamatin (1934) and Schaefer (1936).

Dissociation of the Uncapsulated Variant of Strain XXII.—Two nutrient broth and two serum broth tubes were inoculated with the uncapsulated strain. One of each lot was incubated at 37° C. and the other at 42° C. At intervals a loopful of each culture was streaked on nutrient agar and the form of the developing colonies examined.

The following symbols are used to denote the type of growth:

R = Rough.
S = Smooth.
M = Mucoid.
P = Phantom.
T = Transparent, flat and spreading.
O = Opaque and raised.

The degree of sporulation is indicated thus:—

– No spores.
+ + Occasional free-lying spores.
+ + + + 90 to 100 per cent. free-lying spores.

The following table shows the main types of colonies obtained

TABLE I.

Loopful of broth culture streaked after	Types of colonies which grew in streaks made from uncapsulated avirulent strain grown in			
	Broth at		Serum broth at	
	37 C.	42 C.	37 C.	42 C.
7 days.......	R++++	R++++	R++++	R– RO++
14 days.......	RT– RO++++	RT– RO++++	R++++	RT– RO+
21 days.......	RT– RO++++	RT– RO++	R P	R
28 days.......	RT– RSO++++	RT– RSO++++	RT++ RSO+++ P	R– RO++++ P
50 days.......	R– RSO+++	No growth	R RO++++	

It seems that the rough uncapsulated parent strain split into two main strains, the one having a transparent, flat rough colony and the other a more raised, opaque and somewhat smoother colony. The bacilli in the former did not sporulate or sporulated rarely, whereas the bacilli in the latter sporulated readily.

Further Dissociation of Variants Noted in Table I.—Some of the variants noted above were studied in more detail to see whether further dissociation would occur freely. A rough, transparent non-sporulating colony and an opaque sporulating colony (14 days, column 1, Table I) were seeded into separate tubes of nutrient broth and the cultures which developed streaked at intervals on nutrient agar.

TABLE II.

Loopfuls of broth culture streaked after	Types of colonies which grew in streaks made from broth cultures of	
	Rough transparent non-sporulating variant (RT–).	Rough opaque sporulating variant (RO ++++).
1 day..............................	RT–	RSO++++
18 days.....................	RT	RT– RO++++
25 days......................... ...	RT–	RT– RSO++++
50 days..............................	RT–	RT–

Other rough opaque and rough transparent colonies were grown in broth for periods of 180 days and streaks made from these cultures showed almost the same appearance as streaks made in the experiment noted in Table II. Thus it seems that the RO variant dissociated into RO and RT types while the RT variant did not produce RO forms. This dissociation of the RO colony sometimes occurred as "sector" variation—i.e. sectors of the RT type developed in RO colonies.

It must not be supposed that only these two sorts of colonies appeared during the course of the experiments. Although the pattern of variation was mainly that indicated above yet subsidiary "trends" were frequently seen. The RT colonies, in which the bacilli grew in chains, sometimes showed sectors in which the bacilli grew singly. Such sectors, while still rough, transparent and non-sporulating, could be clearly differentiated from the long chained parent strain. The differences persisted after the variants had been sub-cultured. Occasionally, smooth looking colonies which resembled slightly smooth mucoid anthrax strains were noted. The bacilli, however, were neither capsulated nor virulent.

Continuous Dissociation of a Phantom Variant.—The phantom growth which developed from strain XXII (21 days, column 3, Table 1) was streaked on nutrient agar. After 24 hours a faint lustreless film appeared which showed, under low magnification, a number of minute slightly raised condensations. After a further 24 hours these condensations were plainly visible as raised rough opaque colonies scattered in the film. The phantom growth was subcultured two or three times a week for seven months and always commenced as a thin film with minute condensations which later developed into rough colonies. The rough colonies remained rough on subculture.

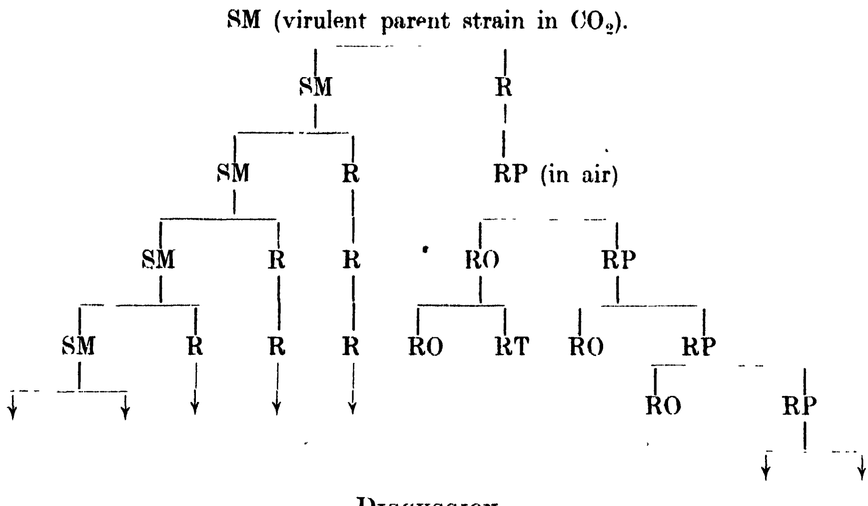
This seemed to be therefore, a clearcut example of continuous variation where the unstable rough phantom continuously reproduced itself and also threw off a relatively stable rough non-phantom daughter strain.

Dissociation of Strain XXXV.

This virulent strain was grown on 50 per cent. serum agar in 20 per cent. carbon dioxide immediately after its isolation. Under these conditions the growth was, as is the rule, smooth mucoid. After 48 hours a rough variant sector was selected and streaked on nutrient agar. This subculture appeared as a thin film dotted with small opaque rough colonies. Selection and subculture of the latter yielded only rough opaque colonies which in their turn gave rise to the rough transparent type. The peculiarity of strain XXXV was the rapidity with which different sets of dissociating colonies were established.

Schematic Representation of Dissociation in Strain XXXV.

All the SM variants were virulent and all others avirulent. The SM—➤R change is naturally only recognizable in carbon dioxide. All the other changes were under ordinary conditions.



Discussion.

Sufficient has been done to show that there are a large number of factors involved in dissociation phenomena and that even relatively debased strains possess great variability. The examples of continuous variation given above and more particularly the example of strain XXXV, emphasize the fluidity of bacterial characteristics and show that a diversity of colony forms can be quickly obtained from apparently homogeneous cultures. Deskowitz and Shapiro (1935) and Deskowitz (1937) suggested that there was only a quantitative difference between unstable (continuous) variation and ordinary variation. The results obtained above with the moderately unstable RO strain support their contention.

Anthrax cultures often show a marked heterogeneity (interesting examples are given by Stamatin 1935a and 1935b) and such bacterial populations are, in general, very sensitive to environmental changes. This sensitivity is partly owing to the absence or rarity of reverse mutations so that an equilibrium during growth is only exceptionally established. As a rule a stable state can only be maintained by active intervention, as for example by frequent subculturing or the use of special media.

During the present work smooth contoured variants sometimes arose from rough parent strains and it seemed that such occurrences were more frequent in "debased" strains (variants which formed neither capsules nor spores and were neither virulent nor able to elicit immunity). Such late appearing smooth forms, although resembling superficially the smooth precursors of the rough variants were in reality entirely different and lacked properties characteristic of the early smooth strains (capsules, spores, virulence). It must be remembered that "rough" and "smooth" are vague, elastic terms and may include a variety of colony types. Such a fortuitous resemblance, the result of a common smooth appearance, must not be allowed to imply either a reversion or a cyclical change.

A consideration of the variants described here and of the wide variety of forms described by other authors emphasizes the difficulty of postulating any cyclical variation in the anthrax bacilli. Most strains, apparently, undergo degradation on artificial media. Capsulated and virulent strains become uncapsulated and avirulent; sporulating strains become non-sporulating. It is impossible to say whether these and the many other variants described are important stages in a life history or reactions to an artificial environment.

Summary.

1. Two virulent anthrax strains incubated in carbon dioxide gave rise to uncapsulated avirulent variants and from the latter a number of other variants were isolated and studied.

2. There was a tendency for sporogenous variants of the uncapsulated strain to produce asporogenous types.

3. On different occasions highly unstable rough phantom variants developed from the uncapsulated strains and gave rise continuously to rough non-phantom daughter strains. These in their turn showed further dissociation.

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Section II.

Parasitology.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 10, *Number* 2, *April*, 1938.
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South African Helminths—Part II.

Some Taenias from Large Wild Carnivores.

By R. J. ORTLEPP, Section of Parasitology, Onderstepoort.

NOTWITHSTANDING the fact that South African large wild carnivores have been collected for museums and zoological gardens for over a century, it is a remarkable fact that up to quite recently no Taenias have been desciibed from these animals. A possible explanation is that collectors for museums have not concerned themselves about the internal parasites of these aniamls, and that those animals which have died in zoological gardens had either not been examined for helminths or their death may have occurred some considerable time after their capture by which time they had probably lost their original infection.

During the short period of the Zoological Survey's operation a few large carnivores have been shot and on examination all weie found to harbour Taenias; in some cases the infection was particularly heavy. This was not surprising as our antelopes, from the region where these carnivores were obtained, were generally infected with " measles " and as these antelopes constitute the natuial food of these carnivores one would naturally expect to find these hosts harbouring Taenias.

The Taenias from three lions, one leopard, one cheetah and one jackal, all obtained by the Zoological Survey are here described; in addition Taenias from a cheetah and a wild dog from South West Africa and Taenias from three jackals from the Orange Free State, Transvaal and Cape Province respectively are also incorporated.

Two species of Taenia have so far been described from our large carnivores, namely *T. regis* Baer, 1923, from a lion and *T. hyaenae* Baer, 1927 from an hyaena. An examination of the writer's material failed to reveal the presence of these two species, neither, except in in three cases, viz. *T. multiceps* and *T. serialis* from jackals and *T. pisiformis* from a wild dog, did the writer's specimens agree with any hitherto described species of Taenia. The absence of *T. hydatigena* was all the more striking because the larval stage of this parasite is a very common parasite in our sheep and goats and has also been found in our antelopes. In consequence the writer

has no other choice but to regard his specimens as representing hitherto unknown species. It may be mentioned that "measles" from antelopes and wild pigs, which the writer had previously examined, had proved not to be identical with the larval Taenias of our domestic pigs, cattle and sheep, namely *Cysticercus cellulosae*, *C. bovis*, *C. ovis* and *Caenurus cerebralis*.

From Lions.

*Taenia bubesei** sp. nov.

This species was represented by many specimens collected together with the following species from a single lion. The strobilae are somewhat contracted and are from 45 to 55 cms. long with a maximum breadth of 6 to 9 mm. according to the state of contraction; there are from 360 to 375 segments in entire specimens; mature segments are about 6 mm. broad and 3 mm. long and ripe segments reach a length of 8 mm. and 4 mm. broad.

The head is about 1·3 mm. broad and the suckers are somewhat rounded measuring 0·34 mm. in diameter. The rostellum is massive and about 0·79 mm. thick; in 15 heads examined it carried 42 to 46 hooks in two circles. The large hooks are from 0·235 to 0·273 mm. long and the smaller hooks 0·136 to 0·18 mm. long from the tip of the blade to the tip of the handle (Fig. 1); the blade is strongly arched and the lateral outline of the handle of the large hook is sinuous.

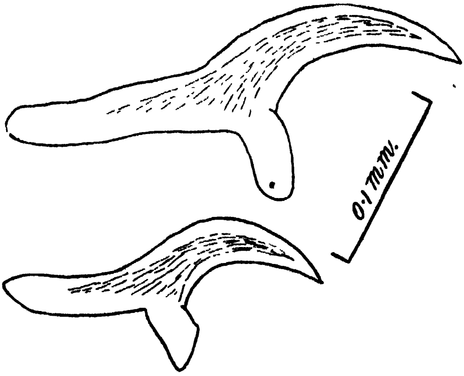

Fig. 1. *T.bubesei* sp. nov. Large and small hooks.

The cuticle is 0·008 to 0·009 mm. thick and is followed by a thin sheet of sub-cuticular transverse muscles 0·012 to 0·015 mm. thick; the longitudinal muscle layer fills practically the whole cortex and is about 0·2 to 0·23 mm. thick; it consists of numerous fibres 0·006 to 0·008 mm. thick irregularly scattered in its outer half and forming small bundles of 5 to 10 fibres each in its inner half (Plate 1, Fig. 1). The inner transverse muscle layer is 0·029 to 0·032 mm. thick. Dorso-ventral muscle fibres are well developed and are especially evident in the medullary parenchyma. Chalk bodies are relatively few in number and are mostly confined to the cortex; they are 0·015 by 0·012 mm. in size and smaller.

* "Bubesi" is Zulu word for "lion".

The ventral excretory canals are large, measuring 0·087 by 0·17 mm. to 0·1 by 0·17 mm. in diameter; they are situated 0·95 to 1·1 mm. from the lateral margins of the segment; the dorsal excretory vessels are situated dorso-laterally to the ventral excretory canals in the inner corner and have a diameter of about 0·009 mm.

The nerve cords are large, about 0·08 by 0·07 mm. in cross section and situated about 0·78 mm. from the edge; the two accessory nerve cords are distinct. The genital ducts pass between the excretory canals and dorsal of the nerve cord.

The genital atria alternate irregularly and in mature segments are situated just anterior to the middle of the segment; they are not very prominent. The atria are fairly large and the genital cones on their floor are inconspicuous. The club-shaped cirrus sac is 0·38 to 0·4 mm. long with a maximum thickness of 0·1 mm; the vas deferens makes a few loose coils in the cirrus sac but after emerging it becomes very intricately coiled (Fig. 2); the testes number between 500 and 600 and they extend backwards to the posterior level of the yolk gland and in some cases even further; none, however, were observed to pass behind this organ; they leave a fairly wide space free of testes round the female genital glands; the aporal and poral testicular groups are united to each by a narrow bridge of testes generally only 2 to 4 testes broad.

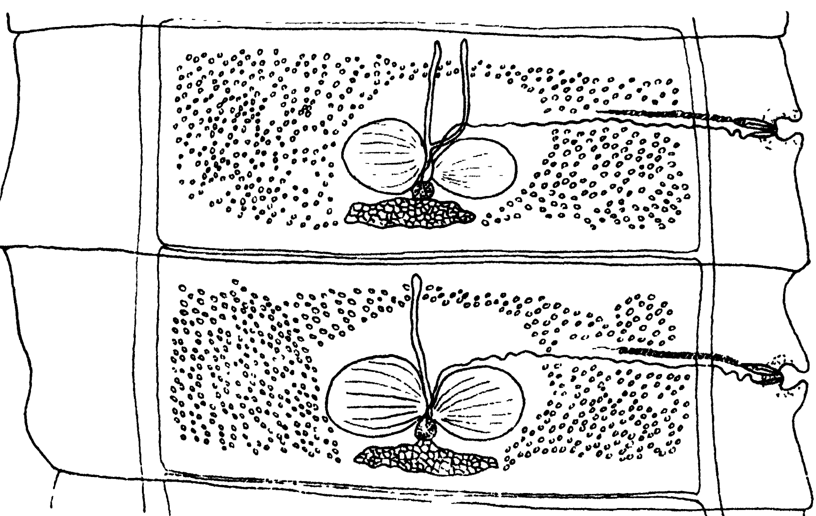

Fig. 2. *T. bubesei* sp. nov. Mature segment.

The vagina opens behind and on the same level as the cirrus sac; its initial portion is slightly enlarged, after which it forms a few large loops; after crossing the excretory canals it is continued inwards as a wavy line skirting the anterior margin of the poral ovarian lobe.

The two ovarian lobes are hemispherical, the aporal lobe being larger than the poral; the yolk gland is reticular and extends almost the width of the ovary. The uterus reaches the anterior edge of the segment and the main lateral branches are few in number, generally only 3 to 5 are present on either side, but occasionally 6 or 7 are present (Fig. 3). In some strobilae (3 out of 7 strained) there was a curious tendency for the main uterine stem to become double in some of its segments; in such cases the lateral branches were limited to the outer margin of each stem. The eggs are numerous, oval, thick-shelled and pitted, measuring 0·039 by 0·031 mm. to 0·042 by 0·032 mm.; the shell is 0·006 mm. thick.

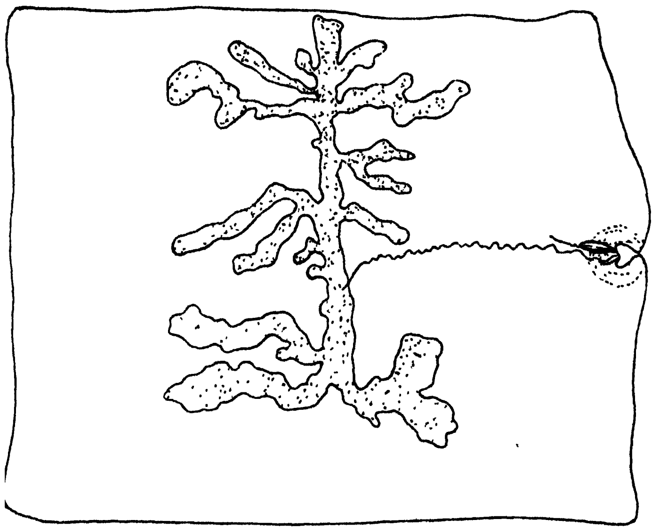

Fig. 3. *T. bubesei* sp. nov. Uterus.

Discussion.—*T. regis* Baer, 1923 is the only cestode described from a lion. The above described species differs from it by its large size, greater number of rostellar hooks which are smaller, greater number of testes which extend to the posterior level of the yolk gland, the wavy outline of the vagina, and the larger number of lateral uterine branches which are not confined to the anterior and posterior ends of the main stem. Its closest relative appears to be *T. pisiformis* (Block, 1780), which species it resembles in that the number of hooks exceed 40 and their size and shape are also very similar, and in that the vagina is wavy; it differs from this species, however, in that the number of hooks always exceed 40, the base of the small hook is not bifid, the cirrus sac does not reach or cross the excretory canals, the testes form a narrow bridge anterior of the ovary, no testes are present behind the yolk gland, and the lateral uterine branches are not so many.

Specific Diagnosis.—Taeniadae reaching a length of 55 cms. by 6-9 mm. broad and having nearly 400 segments; there are 42 to 46 rostellar hooks in two circles; the larger hooks are 0·235 to 0·273 mm. long and the smaller hooks 0·136 to 0·18 mm. long. The

testes number 500 to 600, do not pass round back of yolk gland and form a narrow bridge in anterior portion of segment; they leave a large clear space round the female glands. The cirrus sac does not reach excretory canals. Uterus shows a tendency to be double in its anterior half and the lateral uterine branches generally number three to five on either side, occasionally there may be seven. Eggs oval with pitted shell, measuring 0·039 by 0·031 mm. to 0·042 by 0·032 mm.

Host.—*Leo leo krugeri.* (Rbts.)

Locality.—Transvaal.

Location.—Small intestine.

Types in the Onderstepoort Helminthological collection.

*Taenia gonyamai** sp. nov.

This cestode was collected in association with *T. bubesci* on one occasion and separately on two others. Macroscopically it appeared thinner and paler than the former species, although in section the musculature in these two species appears to be equally developed.

The length of the strobilae reaches 52 cms. with a maximum breadth of 8 mm. in its 3rd and 4th fifth. There are about 260 segments of which the 190th to 220th are mature and these segments are about 4 mm. long by 6 mm. broad; ripe segments become much elongated and may reach a length of 33 mm. by 4 mm. broad.

In twelve scolices the number of hooks varied from 32 to 38 and the large hooks were from 0·188 to 0·209 mm. long and the smaller hooks 0·122 to 0·142 mm. long from the tip of the blade to the tip of the handle (Fig. 4). In practically all the larger hooks the blade and handle were more or less in a straight line and the outline of the handle was smooth except for a distinct notch on its dorsal side.

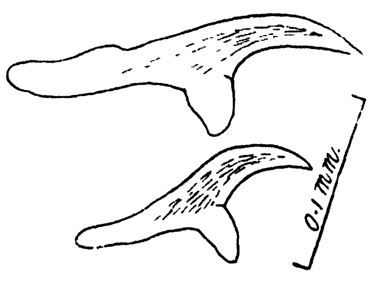

Fig. 4. *T. gonyamai* sp. nov. Large and small hooks.

In transverse sections of mature segments (Plate 1, Fig. 2) the cuticle is 0·008 to 0·01 mm. thick; the underlying transverse muscles form a very thin layer. The longitudinal muscles occupy most of the cortical parenchyma and consist mostly of irregularly scattered muscle fibres of which the innermost are grouped together into

* " Gonyama " is Swazi word for " lion ".

bundles with 2 to 15 fibres each, the majority consisting of 3 to 8 fibres each, each fibre being 0·005 to 0·007 mm. thick. The inner transverse muscles form a band 0·029 to 0·035 mm. thick. Dorso ventral muscle fibres are present and are especially evident in the medulla. Only a few chalk bodies are present and these are practically confined to the cortex; they are oval and measure up to 0·014 by 0·011 mm.

The ventral excretory canals have a diameter of 0·133 by 0·098 mm. to 0·124 by 0·191 mm. and are situated 0·9 to 1·1 mm. from the lateral margin. The dorsal excretory vessel have a diameter of 0·025 to 0·029 mm. and are situated 0·04 to 0·145 mm. on the dorso-lateral corner of the ventral canals. The nerve cord has a diameter of 0·073 to 0·087 mm. and is situated about 0·75 mm. from the lateral margin; the two accessory lateral nerves are distinct.

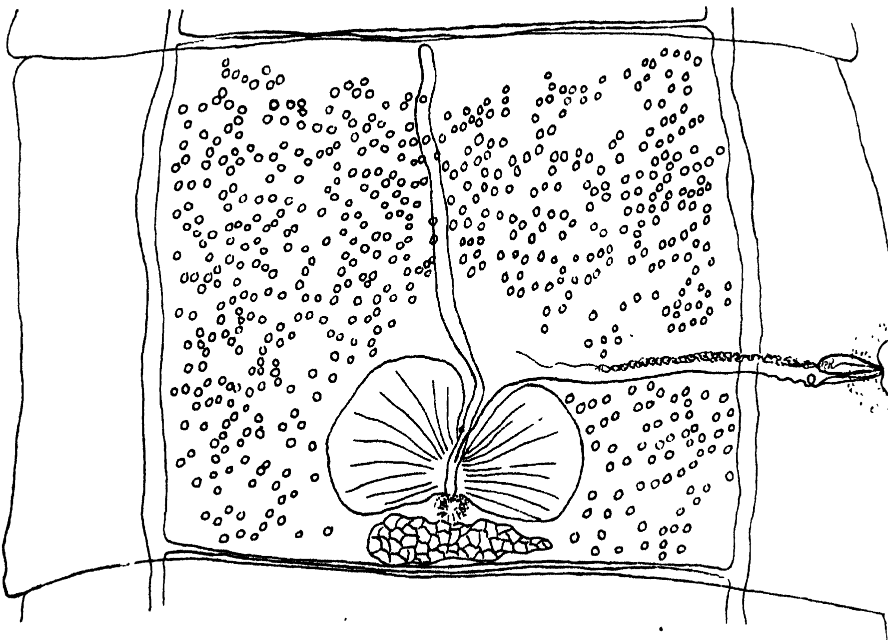

Fig. 5. *T. gonyamai* sp. nov. Mature segment.

The genital atria alternate irregularly and are situated just behind the middle of the segment in mature segments; the atrium is large and deep but not prominent measuring 0·38 mm. across and 0·23 mm. deep; its floor is more or less flat, a prominent genital cone being absent. (Fig. 5.) The cirrus sac is club-shaped and does not reach the ventral excretory canal; it measures 0·4 to 0·44 mm. long with a thickness of 0·12 to 0·15 mm. The cirrus is unarmed and is 0·04 mm. thick at its base. The vas deferens forms a few coils inside the cirrus sac and is intricately coiled before and after crossing the excretory canal; it passes between the excretory canals and dorsal of the nerve. The testes are numerous numbering between

500 and 600; they are arranged in a single layer in the dorsal half of the medulla; they have a diameter of about 0·07 mm; they extend from the posterior level of the yolk gland, just miss the lateral edges of the ovarian lobes, and the poral and aporal testes are united together by a broad bridge of testes in front of the ovary; only a relatively small hemispherical space in front of the ovary is free of testes.

The initial portion of the vagina is slightly inflated and then forms a few conspicuous waves or loops; after crossing the excretory canals its course is straight although in some segments it may be slightly wavy; the genital glands are as found in *T. hydatigena*. The uterus extends through the length of the segment and differs from that of *T. hydatigena* in possessing more lateral branches and generally not showing the massing of uterine branches at its anterior and posterior extremities; these branches number from 14 to 18 on either side (Fig. 6). The eggs are oval, thick-shelled and pitted; they measure 0·029 to 0·031 mm. by 0·032 to 0·04 mm. and the shell is 0·006 mm. thick.

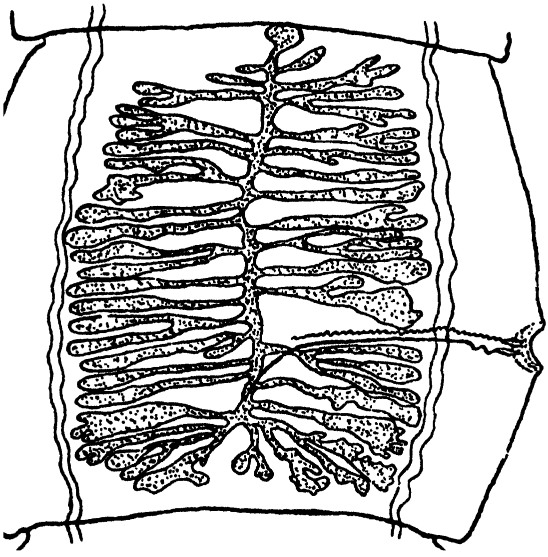

Fig. 6. *T. gonyamai* sp. nov. Uterus.

Discussion.—This species resembles *T. hydatigena* but can be distinguished from it in that the rostellar hooks do not exceed 38 in number, the dorsal edge of the handle of the blade is always notched, the vagina is coiled prior to crossing the excretory canal and there are more uterine branches.

In *T. hydatigena* the blade and handle form an obtuse angle and the outline of the handle is sinuous; although this is their normal appearance the writer found variations in his material of this species showing gradations from such hooks to hooks with the blade almost in a straight line with the handle and the outline of the handle varying from sinuous to smooth with a dorsal notch.

Specific Diagnosis.—Taeniadae reaching 52 cm. in length carrying 32-38 rostellar hooks in two rows; large hooks 0·188 to 0·209 mm. long, smaller hooks 0·122 to 0·143 mm. long; edge of handle of large hooks smooth but carries a dorsal notch. Male and female genitalia as in *T. hydatigena* except that initial portion of vagina is coiled, the uterus has 14 to 18 lateral branches and the eggs are oval, thick-shelled and pitted.

Host.—*Leo leo krugeri.* (Rbts.)

Locality.—Transvaal.

Location.—Small intestine.

Types in the Onderstepoort Helminthological Collection.

From Leopard.

Taenia Ingwei sp. nov.*

This species is represented by two complete worms, 3 fairly long fragments with scolices and four older fragments. The two complete worms are 250 and 270 mm. long and have a maximum breadth of 3·7 and 4·6 mm. respectively; the end segments are bell-shaped and measure 5·5 to 6·0 mm. long by 3 to 3·8 mm. wide at their posterior margins. The whole strobila is hard and is bent in a zig-zag manner; the collector informed the writer that this appearance was also noticed, although not so marked, when these worms were removed alive from the freshly killed leopard.

The head is small and measured from 0·72 to 0·79 mm. across, the four suckers are rounded and not prominent and have a maximum diameter of 0·29 mm; the rostellum measures 0·39 mm. across and carries 32 to 34 hooks in two circlets (Fig. 7); the larger hooks are 0·197 to 0·202 mm. long and the smaller 0·148 to 0·151 mm. The neck is very short, only 0·46 mm. long and is 0·07 to 0·76 mm. broad.

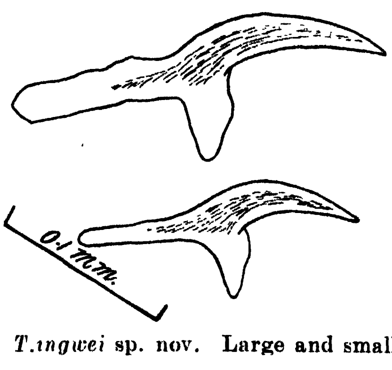

Fig. 7. *T. ingwei* sp. nov. Large and small hooks.

In sections of mature segments (Plate 1, Fig. 3) it is seen that the cuticle is remarkably thick and corrugated and may attain a thickness of 0·035 mm; this is probably the reason for the hardness of the worms and the peculiar shape of the strobila. The subcuticular transverse fibres form a layer 0·009 mm. thick. The cortex

* "Ingwe" is Zulu word for "leopard".

has a thickness of about 0·23 mm. and its inner portion, for a thickness of about 0·175 mm., is occupied by the longitudinal muscles; these are well developed and consist mostly of muscle bundles there being very few isolated fibres towards the periphery; the bundles are crowded together and each consists of 20 to 30 fibres each having a thickness of 0·004 to 0·006 mm. The inner transverse muscles are well developed and form a layer 0·04 to 0·045 mm. thick. The medulla has a thickness of 0·174 to 0·188 mm. and is traversed by numerous stout dorso-ventral muscle fibres which branch outwards through the cortex. Both the cortex and medulla are crammed with numerous oval chalk bodies measuring 0·024 by 0·015 mm. and less. The ventral excretory canals are situated 0·85 to 0·87 mm. from the edge of the segment and have a diameter of 0·104 by 0·075 mm. to 0·133 by 0·087 mm.; the dorsal excretory vessels are situated some 0·07 mm. from the ventral excretory canal in its inner dorso-lateral corner; they have a diameter of 0·015 to 0·023 mm. The lateral nerve is 0·072 to 0·087 mm. from the ventral excretory canal and has a diameter of about 0·06 mm. The genital ducts pass between the excretory canals and dorsal of the nerve.

The genital atria alternate irregularly, are prominent and fairly deep; they are about 0·32 mm. across by 0·26 mm. deep and at their base there is a dome-shaped genital cone.

The cirrus sac is club-shaped, reaches but does not cross the excretory canals; its maximum length is 0·435 mm. and maximum thickness 0·087 mm. The vas deferens forms a few coils in the cirrus sac and after passing the excretory canals become intricately coiled. The denseness of the cuticles makes it difficult to arrive at a correct estimate of the number of the testes, but there appear to be between 400 and 500; they leave a clear space round the female glands and they extend backwards to the level of the yolk gland but not behind; the testicular bridge in front of the ovary occupies about half of the length of the segment in front of the female glands.

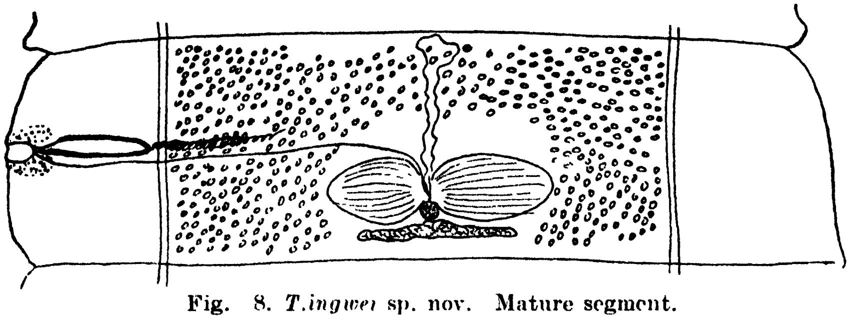

Fig. 8. *T. ingwei* sp. nov. Mature segment.

The vagina opens on the genital cone behind the cirrus sac and is straight i.e. no waves or coils are present in its transverse course (Fig. 8). The female glands show no special characteristics. The uterus carries 6 to 10 lateral branches (Fig. 9) each of which may terminate in several club-shaped secondary branches; the most anterior branch is massive and gives off several short club-shaped branches on its anterior face. The eggs are oval and the 0·006 mm. thick shell is pitted; they are from 0·035 to 0·038 mm. long by 0·028 mm. in diameter. The hexacanth hooks are 0·012 mm. long.

Discussion.—The size and number of the hooks may be considered to fall into the range of those of *T. hydatigena*, *T. gonyamai* and *T. hlosei;* however, its smaller strobila with its peculiar shape, its much thickened cuticle and the greater development of its musculature, easily serve to distinguish it from these three species.

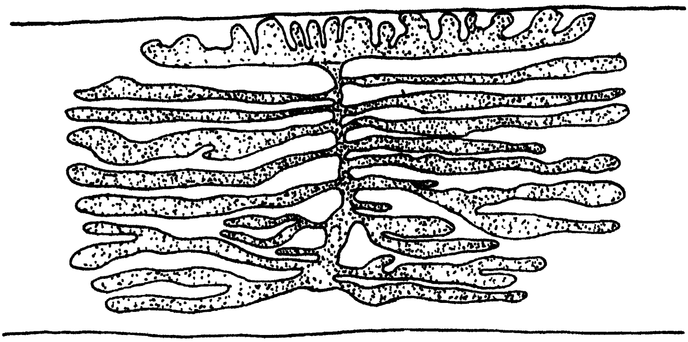

Fig. 9. *T. ingwei* sp. nov. Uterus.

Specific Diagnosis.—Taeniadae reaching a length of 270 mm. by 4·6 mm. broad. Strobila hard and bent zig-zag even in living specimens. Rostellum carries 32 to 34 hooks in two circlets; large hooks 0·197 to 0·202 mm. long small hooks 0·148 to 0·151 mm. long. Neck short and thick. Cuticle thick and musculature well developed. Numerous chalk bodies in parenchyma. Genital atria large and prominent; about 400-500 testes not extending backwards beyond vitelline gland, leaving a fairly wide space round ovary and forming a fairly wide bridge anterior of female glands. Vagina straight; uterus with 6 to 10 lateral branches and anterior branch massive with several anterior club-shaped secondary branches. Egg oval and shell pitted, 0·035 to 0·038 mm. long by 0·028 mm. in diameter.

Host.—*Panthera pardus.*

Location.—Small intestine.

Locality.—Northern Transvaal.

Types in the Onderstepoort Helminthological Collection.

From Cheetah.

*Taenia Hlosei** sp. nov.

This species was represented by two almost complete worms and several fragments, seven of which carried scolices. The almost complete specimens were 325 and 400 mm. long and had a maximum breadth of 9 mm. and 8·5 mm. respectively, their end segments measured 6·5 mm. broad by 4·5 mm. long and 5·5 mm. broad by 5 mm. long respectively. As some of the fragments contained longer end segments, reaching 7 mm. long by 4·5 mm. broad and as these were bell-shaped whereas those of the two measured specimens were rectangular, it is certain that in complete specimens the lengths would be some 50 mm. longer than those mentioned above.

* "Hlosi" is Zulu word for "cheetah".

The scolex is large, 1 to 1·1 mm. across and carries 4 prominent rounded suckers having a diameter of 0·5 mm.; the rostellum is 0·45 mm. thick and carries a double circlet of 36 to 40 hooks. The larger hooks are 0·209 to 0·215 mm. long and the shorter ones 0·145 to 0·151 mm. long (Fig. 10). The upper margin of the handle of the large hook is generally sinuous, but it may vary from being practically smooth to possessing a distinct notch.

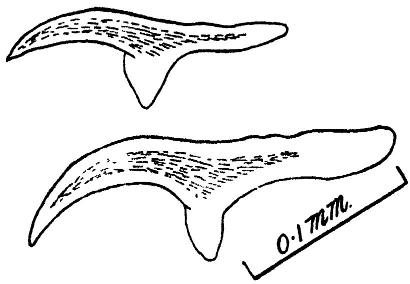

Fig. 10. *T. hlosei* sp. nov. Large and small hooks.

The neck is short and broad and measures about 1 mm. long by 0·9 mm. broad.

In sections of mature segments (Plate 1, Fig. 4) the cuticle has a thickness of 0·017 to 0·023 mm. and is followed by a thin layer of subcuticular transverse muscle fibres; the cortex is 0·26 to 0·29 mm. thick of which the inner half is traversed by the longitudinal muscle layer; this layer is composed internally of small irregularly scattered muscle bundles containing 5 to 10 fibres each, each fibre having a diameter of 0·004 to 0·006 mm.; externally this layer is composed of scattered single muscle fibres. The inner transverse muscles are well developed and form a layer 0·029 to 0·035 mm. thick. The medulla has a dorso-ventral thickness of 0·3 to 0·43 mm. Both the cortex and medulla are closely packed with numerous oval chalk bodies reaching a maximum size of 0·02 by 0·25 mm.

The ventral excretory canals are large and have a diameter of 0·145 by 0·116 mm. to 0·215 by 0·104 mm. and are situated 0·8 to 0·9 mm. from the edge of the segment. The dorsal excretory canal has a darker staining wall, 0·017 to 0·023 mm. in diameter and is situated 0·03 to 0·058 mm. from the ventral excretory canal on the inner dorso-lateral corner. The nerve is prominent and measures about 0·09 mm. across. The genital ducts pass between the excretory canals and dorsal of the nerve.

The genital atria are prominent and situated just behind the middle of the segment; the atria have a depth of about 0·35 mm. and are about 0·23 mm. across; there is a prominent genital cone at their base.

The cirrus sacs are club-shaped and extend to but do not cross the excretory canals; they are from 0·475 to 0·487 mm. long with a maximum thickness of 0·133 mm. The vas deferens forms a few coils in the cirrus sac and after crossing the excretory canals it becomes very densely coiled. The testes appear to be in the vicinity

of 400 to each segment and generally do not extend beyond the posterior level of the vitelline gland; occasionally, however, a few may be present further back. They leave a large clear space round the female glands and are more concentrated laterally; they form a fairly wide bridge of loosely scattered testes in the uterine field; they are arranged in a single layer in the dorsal half of the medulla and have a dorso-ventral diameter of 0·133 to 0·145 mm.

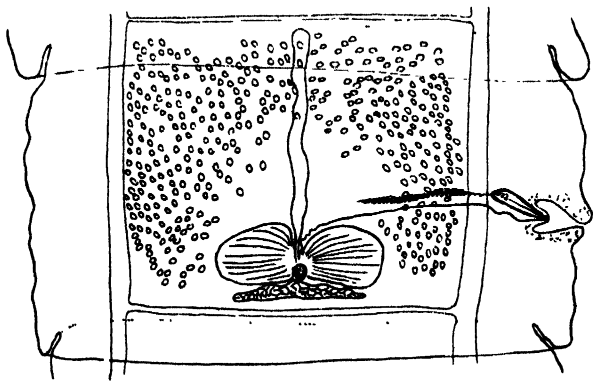

Fig. 11. *T. hlosei* sp. nov. Mature segment.

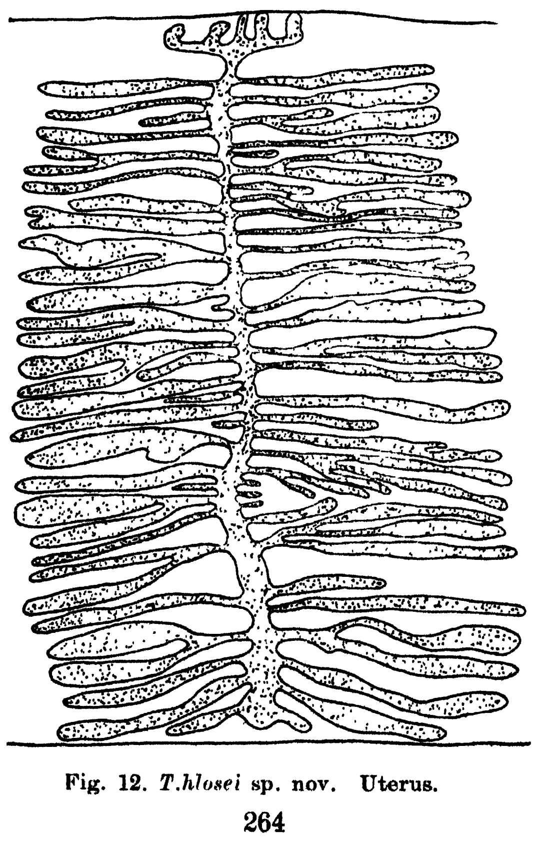

Fig. 12. *T. hlosei* sp. nov. Uterus.

The vagina opens behind the cirrus sac on the genital cone; its course is practically straight, although in some segments it may be very slightly wavy before crossing the excretory canals (Fig. 11). The ovary and vitellarium show no special differentiating characters. The uterus has from 20 to 30 lateral branches (Fig. 12) arising from the main uterine stem; generally the number is about 24; they are long and narrow and are closely packed together. The eggs are oval with pitted shell and measures 0·032 by 0·026 mm. to 0·035 by 0·029 mm. and the shell is 0·0045 to 0·006 mm. thick.

Discussion.—The size of the large hooks falls in the range of those of *T. hydatigena*, *T. ingwei* and *T. gonyamai*, but the small hooks are generally slightly larger than those of the latter species. The much greater number of uterine branches easily distinguishes it from *T. ingwei* and *T. hydatigena* and from the latter species it also differs by its smaller strobila and numerous chalk bodies. Its closest relative is *T. gonyamai* from which it differs, however, by having more uterine branches, less testes, better developed musculature and numerous chalk bodies.

Specific Diagnosis.—Taeniadae reaching a length of about 450 mm. by 9 mm. broad and provided with 36 to 40 rostellar hooks in two circlets; the larger hooks measuring 0·209 to 0·218 mm. long and the smaller 0·142 to 0·151 mm. The neck is relatively short and thick and the parenchyma is thickly crammed with numerous chalk bodies. There are about 400 testes, concentrated laterally, leaving a large clear space round the female glands, generally not extending behind the vitelline gland, and forming a fairly broad bridge in the anterior half of the segment. Th vagina is practically straight and the uterus carries from 20 to 30 lateral branches. The eggs are oval and possess a pitted shell; they measure 0·032 to 0·035 mm. long by 0·026 to 0·029 mm. in diameter.

Host.—*Acinonyx jubatus jubatus* (Erxl.)

Location.—Small intestine.

Locality.—Northern Transvaal.

Types in the Onderstepoort Helminthological collection

Taenia acinomyxi sp. nov.

Only one specimen of this species was obtained; it was 350 mm. long and 3 to 3·2 mm. broad in its posterior half; the strobila is thick and oval in cross section and has a thickness of 2 to 2·2 mm. in mature and older segments. The posterior margin of each segment is raised so that the strobila appears serrated.

The head is 0·9 mm. broad and the rounded suckers have a diameter of 0·35 mm.; the rostellum is 0·435 mm. thick and carries a double circlet of 38 hooks; the large hooks are 0·218 to 0·227 mm. long and the upper edge of the handle is sinuous; the small hooks are 0·128 to 0·136 mm. long (Fig. 13).

The cuticle is 0·015 mm. thick and the following subcuticular transverse muscle fibre layer is 0·014 mm. thick (Plate 1, Fig. 5); the cortex has a thickness of 0·56 to 0·59 mm. and the longitudinal

muscles are distributed practically throughout its whole thickness. The longitudinal muscles, just external of the inner transverse muscles, form a definite layer of small bundles, each containing 5 to 10 fibres, the layer being composed of a single sheet of bundles; a clear space separates this layer from the rest of the muscle fibres, which are irregularly scattered through the rest of the cortex and are composed of small pillars of fibres; generally each of these pillars is built up of 2 to 5 fibres placed one above the other, only occasionally are two fibres found side by side. The fibres have a diameter of 0·007 to 0·008 mm. The inner transverse muscles form a layer 0·052 to 0·058 mm. thick and the medulla is from 0·79 to 0·82 mm. thick. Numerous chalk bodies are present, but are slightly more numerous in the cortex; they are oval and reach a size of 0·02 by 0·013 mm. The dorso-ventral musculature is well developed.

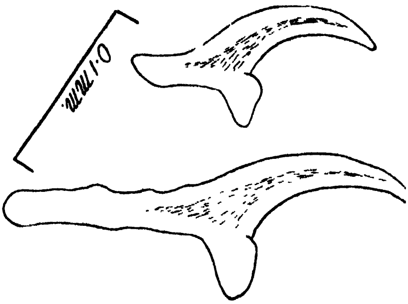

Fig. 13. *T. acinonyxi* sp. nov. Large and small hooks.

The excretory canals are large, the ventral being 0·162 by 0·08 mm. to 0·19 by 0·085 mm. in diameter and the dorsals have a diameter of 0·029 to 0·03 mm. The former are situated 0·4 to 0·46 mm. from the lateral margins and the dorsal vessels are just inside the ventral vessels in the plane of the testes. The nerve, which is 0·087 mm. high and 0·029 mm. broad, lies just lateral of the ventral excretory vessels. The transverse connections of the ventral vessels are large, having practically the same dimensions as the main vessels.

The genital ducts pass between the excretory vessels but dorsal of the nerve.

The genital atria alternate irregularly and are situated just behind the middle of the segment in mature segments and at the junction of the second and last thirds in the oldest segments present. They are somewhat funnel shaped with a maximum cross diameter of 0·12 mm. and 0·29 mm. deep. They are not prominent. Mature segments are 2 to 2·2 mm. long and 3·2 mm. broad. The cirrus sac reaches but does not cross the excretory canals and in mature segments is somewhat club-shaped up to 0·335 mm. long and 0·125 mm. thick; its position is not transverse but it lies in a plane extending diagonally dorsalwards towards the dorsal excretory vessel. The vas deferens makes a few coils in the cirrus sac and immediately after emerging becomes densely coiled dorsal of and beyond the ventral excretory canal (Fig. 14). There are from 250 to 300 testes arranged

in a single layer in the dorsal half of the medulla; they are dorso-ventrally elongated measuring 0·175 to 0·19 mm. deep by about 0·35 tot 0·43 mm. broad. The testes form a large lateral band on either side of the ovary and some testes even lie dorsal of its lateral margins; they extend backwards beyond the posterior level of the yolk gland but are not present behind it; they encroach into the zone of the vagina and vas deferens but leave a large open space in front of the ovary, and the two lateral bands are joined towards the anterior margin of the segment by a narrow bridge of only two to three testes. The vagina makes a few conspicuous loops prior to crossing the excretory canals after which it passes inwards and is regularly convoluted. The ovary, shell and yolk glands show no

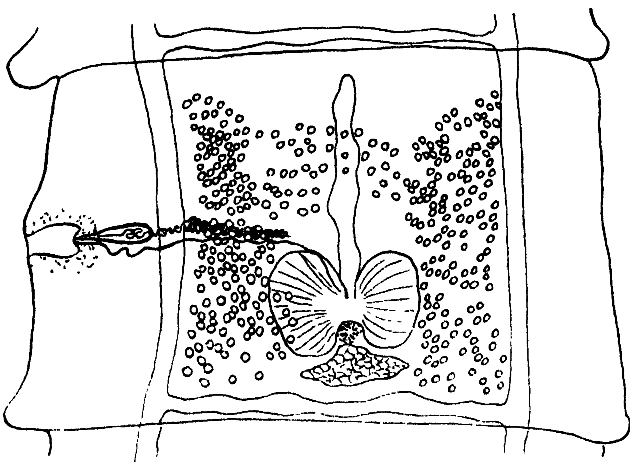

Fig. 14. *T. acinomyxi* sp. nov. Mature segment.

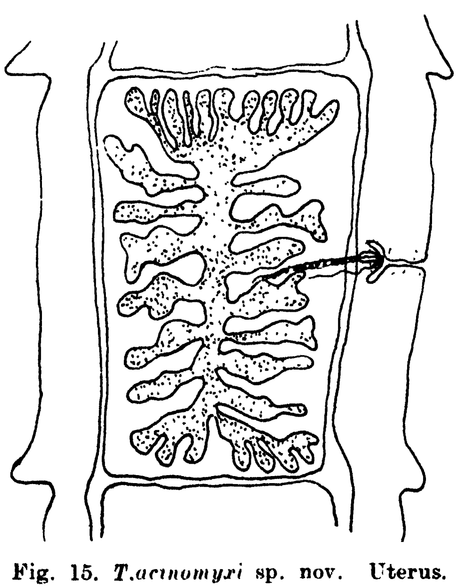

Fig. 15. *T. acinomyxi* sp. nov. Uterus.

special characteristics. The oldest segments are 3·3 mm. long by 2·5 mm. broad, but as they do not contain ripe eggs it is probable that ripe segments will be longer. The uterus has 8 to 10 branches (Fig. 15).

Discussion.—The number and size of the rostellar hooks and the number of uterine branches appear to ally this species to *T. hydatigena* from which it can, moreover, be easily distinguised by its firmer and oval strobila, smaller number and different arrangement of its testes and by the different arrangement of its longitudinal muscles. It differs from *T. hlosei* sp. n., from the same host, by the external shape of its strobila, the different distribution of its longitudinal muscles, its generally longer large hooks and smaller small hooks, in that its lateral uterine branches are considerably less in number, and it also has fewer testes arranged differently.

Specific Diagnosis.—Taeniadae having a firm and oval strobila reaching a length of 35 cms. and possibly over. Rostellum with 38 hooks, in two circles; the larger hooks 0·218 to 0·227 mm. long and the small hooks 0·128 to 0·136 mm. long; handle of large hook with sinuous dorsal border; 250 to 300 testes extending from behind level of yolk gland and forming two lateral bands united anteriorly by a narrow bridge 2 to 3 testes wide; they overlap lateral margins of ovary and leave large clear space in front of female glands. Cirrus sac reaches but does not cross excretory canals; uterus with 8 to 10 lateral branches; longitudinal musculature consists of a single layer of bundles external of inner transverse muscles and of numerous small pillars of fibres scattered through cortex. Numerous chalk bodies present.

Host.—*Acinonyx jubatus jubatus* (Erxl.)

Location.—Small intestine.

Locality.—South West Africa.

Types in Onderstepoort Helminthological Collection.

From Jackal.

Taenia jakhalsi sp. nov.

Four specimens of this species were available for study; the material was killed and fixed with slight stretching in formalin. The longest specimen measured 550 mm. in length with a maximum breadth of 4 mm.; the end segments reach a length of 4·6 mm. by 3 mm. broad and are roughly rectangular in shape.

The scolex is from 0·922 to 0·956 cc. broad and the four suckers are rounded and have a diameter of 0·371 to 0·394 mm. The rostellum is 0·315 to 0·405 mm. across and carries a double circlet of hooks; the four scolices have 30, 32, 32 and 32 hooks each. The large hooks are from 0·195 to 0·22 mm. long and the small 0·131 to 0·142 mm. long (Fig. 16); the blade of the large hooks meets the axis of the handle at an obtuse angle and the upper edge of the handle is slightly sinuous. The scolex is followed by a neck which is from 0·79 to 1·01 mm. long by 0·56 to 0·7 mm. broad.

In mature segments the cuticle is from ·012 to 0·014 mm. thick and is followed by a layer of subcuticular transverse muscle fibres 0·007 mm. thick (Plate 1, Fig. 6). The cortex is about 0·24 mm. thick and the longitudinal muscle fibres are scattered through practically its whole thickness; these muscles consist internally of small bundles containing 3 to 7 fibres each, although occasionally a bundle is present which may contain up to 10 fibres; towards the periphery there are only irregularly scattered single fibres. The fibres have a cross section of 0·007 to 0·01 mm. The inner transverse muscles form a layer 0·015 to 0·017 mm. thick. Dorso-ventral muscle fibres are well developed. The parenchyma of both the cortex and the medulla is thickly crammed with numerous chalk bodies reaching 0·012 by 0·02 mm. in size. The excretory system is remarkable for the almost complete suppression of the dorsal excretory canals, whose position is, however, indicated by a small patch of darker staining cells; where traces of this canal are seen then its diameter does not exceed 0·003. The ventral excretory canal is large and has a maximum diameter of 0·223 by 0·145 mm. and is situated 0·48 to 0·5 mm. from the lateral margin. The nerve measures about 0·06 by 0·035 mm. across and lies just lateral of the excretory canal. The genital ducts pass between the excretory canals and dorsal of the nerve

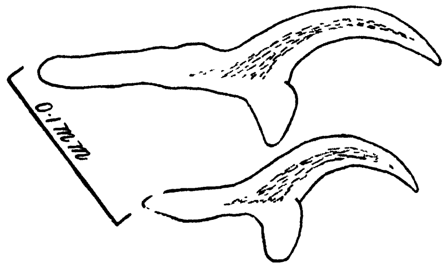

Fig. 16. *T. mkhalsi* sp. nov. Large and small hooks.

The irregularly alternating genital atria are fairly prominent and are up to 0·23 mm. deep by 0·12 mm. across. The cirrus sac is club-shaped and crosses the ventral excretory canal but does not extend inwards beyond it (Fig. 17); it is 0·45 to 0·464 mm. long with a maximum thickness of 0·133 mm. The vas deferens is coiled inside the cirrus sac and on emerging is thrown into numerous large loops. The testes, of which there appear to be between 400 and 500, are generally arranged in a single sheet in the dorsal half of the medulla; occasionally, however, two testes may be seen one above the other; they form two prominent lateral bands extending from the posterior level of the yolk gland to the anterior margin of the segment; these two bands are joined together by a narrow bridge of testes, 2 to 5 testes broad, extending along the anterior margin of the segment; a large space in front of the female glands is left free of testes.

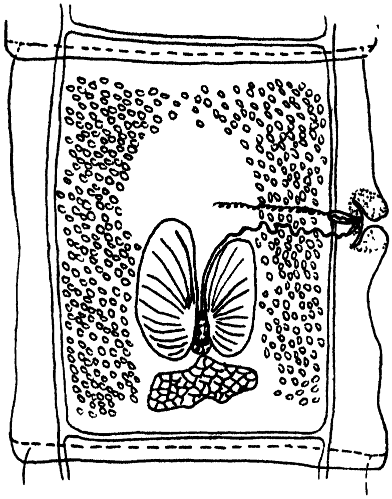

Fig. 17. *T. jakhalsi* sp. nov. Mature segment.

The vagina opens on the same level as but behind the cirrus sac on the dome-shaped genital cone; it is thrown into a few loops prior to crossing the excretory canals after which it passes inwards forming conspicuous convolutions through its whole course; as a rule it just misses the anterior margin of the poral ovarian lobe but in some cases it may cross over this lobe as in *T. ovis*. The ovarian lobes are rounded and the yolk gland is as broad as the ovary. The uterus carries 6 to 10 main lateral branches each of which may give off 2 to 4 secondary branches. The eggs are still immature.

Discussion.—The narrow testicular bridge and the wavy vagina allies this species to *T. bubesei* described above from which it differs, however, by its smaller number of rostellar hooks which are also smaller in size, by its greater number of uterine branches, in that the cirrus sac crosses the ventral excretory canal and in that the dorsal excretory canal is very small or suppressed. The number of hooks, their size and shape, show some similarity to those of *T. hydatigena*, but the arrangement of the testes in this species is quite different.

Specific Diagnosis.—Taeniadae reaching a length of 550 mm. with head less than 1 mm. broad; rostellum with 30 to 32 hooks in two circlets; large hooks 0·195 to 0·22 mm. long, small hooks 0·131 to 0·142 mm. long. Neck about 1 mm. long. Dorsal excretory vessel very small or suppressed; ventral excretory vessel large. Longitudinal musculature of small bundles of generally 3 to 7 fibres each scattered in inner portion of cortex, and from centre of cortex outwards scattered single fibres only. Testes number 400 to 500, generally in a single layer, occasionally 2 testes deep; testes leave a large clear space in front of female glands and form a narrow bridge towards anterior margin of segment. Cirrus sac crosses ventral excretory canal but does not extend inwards beyond it.

Vagina forms large loops before crossing excretory canals, afterwards wavy; it may pass over the edge of the poral ovarian lobe. Uterus with 6 to 10 lateral branches. Eggs immature.

Host.—Thos mesomelas mesomelas. (Schreber.)

Location.—Small intestine.

Locality.—Umtata, Cape Province.

Types in Onderstepoort Helminthological Collection.

*Taenia pungutchui** sp. nov.

Fragments which appear to belong to six different worms were present; unfortunately there were no heads, neither were there any ripe segments contained mature eggs. The longest fragment present, which had only its head and neck missing, was 52 mm. long; its maximum breadth was 3·6 mm. and its end segment was 1·46 mm. long by 2·5 mm. broad. A mounted fragment 35 mm. long had 39 segments, its posterior-most segment had already lost its male and female glands but the eggs contained in the uterus were still very immature; in the anterior-most segment the genital glands were just appearing and their ducts were quite distinct. It would thus appear that fully mature worms may reach a length of about 70 mm. or slightly more and the number of segments probably less than 100.

The excretory system consists of a pair of large and lateral ventral canals joined to each other by a transverse canal at the posterior end of each segment; they have a diameter of 0·093 by 0·075 mm. and are situated about 0·55 mm. from the edge of the segment. The dorsal excretory canals are very small and have a thickened wall; they have a diameter of about 0·006 mm. and are situated dorso-lateral and to the inner side of the ventral canals; in sections of two worms they occupied this position, but in sections of a third the dorsal excretory canal of the left side was ventro-lateral and to the inner side of the ventral canal; in the two normal worms the genital ducts passed over the ventral excretory canals and nerve and under the dorsal excretory canal and in the abnormal specimen this was also the case on the right side but on the left side the genital ducts passed over the dorsal excretory canal and under the ventral excretory canal and nerve. The lateral nerve cords are large and conspicuous, measure 0·06 mm. by 0·032 mm. in section and are situated just lateral of the ventral excretory canals and about 0·5 mm. from the edge of the segment.

The cuticle is thick measuring 0·015 to 0·023 mm. in thickness; the longitudinal muscles are fairly well developed (Plate 1, Fig. 7) and occupy an area 0·15 mm. thick in the cortical parenchyma; they consist of irregularly scattered muscle bundles containing up to 25 fibres each; the larger bundles are more internal in position and the smaller ones towards the periphery; the muscle fibres have a cross section of 0·004 to 0·005 mm. A distinct layer of transverse fibres

* "Pungutchu" is Zulu word for "jackal".

about 0·025 mm. thick separates the cortex from the medulla. Dorso-ventral muscle fibres are well developed especially in the medulla. A few round to oval chalk bodies 0·012 mm. to 0·011 by 0·014 mm. in diameter are present in the cortex.

The genital atria alternate irregularly, are very prominent and are situated in the middle or just anterior of the segment margin; they are somewhat cup-shaped, about 0·2 mm. wide and 0·175 mm. deep (Fig. 18). The base of each atrium is raised to form a small cone on the top of which the openings of the male and female ducts are placed.

The cirrus sac is club-shaped, 0·32 to 0·38 mm. long, with a maximum diameter of 0·07 to 0·08 mm.; it does not reach the ventral excretory canal; the cirrus is small and unarmed; the vas deferens forms a few coils in the cirrus sac and after emerging from it it is thrown into numerous dense coils before and after passing the excretory canals. The testes number about 200 to 250 and have a diameter of 0·09 to 0·095 mm.; they are arranged in two layers and there are about 100 on the poral and the rest on the aporal side. They occupy practically the whole area between the excretory canals not occupied by the female glands, extending backwards to the posterior level of the yolk gland and lying close up to the lateral margins of the ovary; the only area clear of testes is a hemispherical area anterior of the ovary; a narrow bridge of testes, two to four broad, joins the aporal to the poral testicular group anterior of this clear area.

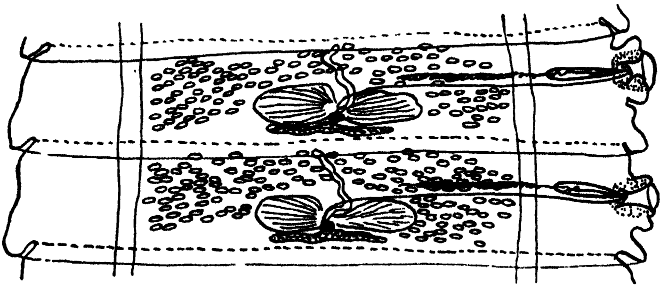

Fig. 18. *T. pungutchui sp. nov.* Mature segment.

The external opening of the vagina is on the same level but behind that of the cirrus sac; it passes inwards in a straight line no convulsions or waves being formed during its course; it just skirts the anterior margin of the poral ovarian lobe and then passes obliquely backwards; sometimes it may cross the anterior margin of the ovary as in *T. ovis*. The poral ovarian lobe is smaller than the aporal lobe and both have the outline of a truncated oval. The shell gland almost fills the vertical space of the medulla between the circular muscles and behind the ovary; it is about 0·2 mm. deep by 0·145 mm. wide and lies between the ovary and yolk gland. The uterus extends to the anterior margin of the segment and in the oldest segments available it was provided with 8 to 10 lateral branches on either side (Fig. 19).

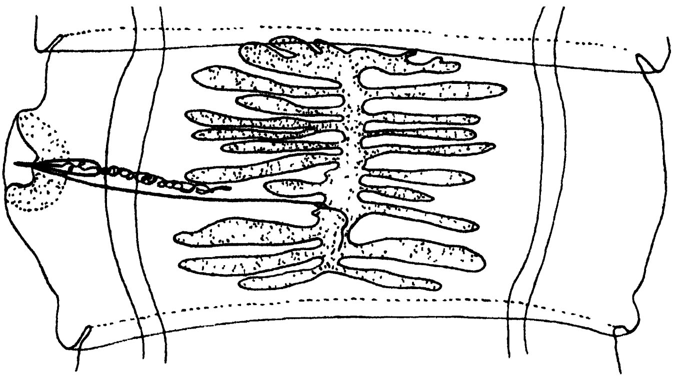

Fig. 19. *T. pungutchui* sp. nov. Uterus.

Discussion.—Notwithstanding the fact that this material is incomplete, there are, however, sufficient characters to distinguish it from the known species of *Taenia*. Such characters are the thick cuticle, relatively short strobila, relatively few testes forming a narrow bridge in front the female glands and arranged in a double layer in the medulla and the moderate number of lateral uterine branches. The thick cuticle, short strobila and number of uterine branches appear to ally it to *T. ingwei* described above, but it may be distinguished from this species by its smaller number of testes arranged in a double layer in the parenchyma and forming a narrower bridge in front of the ovary; and in that its longitudinal musculature is not so well developed, the bundles being fewer and more scattered, and that it has only a few chalk bodies.

Specific Diagnosis.—Taeniadae which may possibly attain a length of 70 mm. and be built up of about 100 segments. Cuticle relatively thick and musculature fairly well developed; chalk bodies small in number. Genital atria very prominent. About 200-250 testes forming a narrow bridge in front of female glands and arranged in two layers in the medulla. Vagina more or less straight; uterus with 8 to 10 lateral branches.

Host.—*Thos mesomelas mesomelas.* (Schreber).

Location.— Small intestine.

Locality.— Northern Transvaal.

Types in the Onderstepoort Helminthological Collection.

Taenia multiceps Leske, 1780.

A large number of specimens of this species was obtained from an undetermined jackal (*Thos mesomelas?*) killed by a farmer in the Philippolis District of the Orange Free State. This particular farmer had suffered severe losses from gid in his sheep and as he had no dogs on his farm he could not account for the infection; however, an intensive search revealed the presence of a few jackals which on being killed were found to harbour this tapeworm. The strobila are short, reaching a maximum length of only 160 mm. but this may be

due to the very heavy infestation of the host; otherwise the writer's specimens agree very well with Hall's (1919) description except that the reflexed loop of the vagina, before crossing the excretory canals, was seldom seen.

Taenia serialis (Gervais, 1847) Baillet, 1863.

A few specimens attaining a maximum length of 150 mm. were obtained from a jackal killed in the Amsterdam district of the Transvaal. In addition numerous specimens were also obtained by experimental feeding of a dog with the larval stages obtained from a hare.

Wild Dog.

Taenia pisiformis (Block, 1780) Gmelin, 1790.

One specimen of this species was obtained from a wild dog (*Lycaon pictus*?) from South West Africa. It carried 36 hooks, the large hooks being 0·22 mm. long and the small hooks 0·15 mm. long; there were between 400 and 500 testes which passed and joined behind the yolk gland. The cirrus sac crossed the ventral excretory canal and the uteri had 10 to 12 uterine branches. The presence or absence of a vesicula seminalis was not determined.

Resume.

Seven species of *Taenia*, hitherto unrecorded, are here described, namely *T. bubesei* and *T. gonyamai* from lions, *T. ingwei* from a leopard, *T. hlosei* and *T. acinonyxi* from cheetahs and *T. jakhalsi* and *T. pungutchui* from jackals; in addition the species *T. multiceps* and *T. serialis* are recorded from jackals and *T. pisiformis* from a wild dog.

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Section III.

Mineral Metabolism and Deficiency.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 10, Number 2, April, 1938.

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The Assimilation of Calcium and Phosphorus by the Growing Bovine.

by J. S. OTTO, Div. of Biochemistry, Onderstepoort.

I. INTRODUCTION.

SINCE the classical studies of Lehmann (1858), in which he demonstrated the absolute necessity of minerals in a ration, the various aspects of mineral metabolism have developed into a complex nutritional problem. Of all the minerals essential for growth and well-being of the organism, calcium and phosphorus have received the most attention. These two minerals, calculated as their oxides, comprise 95 per cent. of the total mineral content of the skeleton.

Either an imbalance under certain conditions or a deficiency of these minerals, which may be found in many food-stuffs, will greatly impair normal growth and development.

The natural pastures in most subtropical climates where the rainfall is unevenly distributed tend to contain insufficient phosphorus for the normal growth in animals, especially at certain times of the year when young actively growing pasture is not available. Under such conditions phosphorus deficiency is usually an acute problem in animal industry. Although a straight calcium deficiency is less likely to occur in animals kept on pasture (for the calcium content of pasture does not vary appreciably with its growth), it may be introduced in stables, where animals are fed on high concentrates, which are low in calcium. Such conditions are known to occur where the deficiency is so acute as to produce osteodystrophic diseases as for instance osteodystrophia fibrosa in horses.

Theiler *et al* (1924, 1927), Henrici (1928, 1930) and du Toit *et al* [1932, 1935, 1935 (*a*)] have established the fact that the pastures of the Union of South Africa are generally low in phosphorus. Orr (1929) showed that the position was equally true for some of the pastures of most countries. It is by no means uncommon for the grazing animal to have in its feed a $CaO : P_2O_5$ ratio of 8 : 1 during winter. Indeed a considerably wider ratio sometimes exists. Hart, Guilbert and Goss (1932) give variations of ratios from unity to 39 : 1.

Under the above grazing conditions of abnormal ratios, animals may show anorexia (Huffman *et al*, 1933), perverted appetite (Green, 1925), soft and porotic bones (Becker *et al* 1933), unthriftiness (Sheehy and Senior, 1930), bent leg (Elliot and Crighton, 1926) and abnormal growth and development (Eckles and Becker, 1926, and

Theiler *et al*, 1927). In order to compensate for the preponderence of calcium over phosphorus, or the deficiency of phosphorus, supplementary feeding of the latter element, in the form of phosphates, is usually resorted to, with markedly beneficial results.

Several authors have expressed regret that data pertaining to the actual mineral requirements for growth are so limited. This lack of information may partly be ascribed to the fact that the availability of minerals differ in various feeding stuffs. The form in which the elements under consideration occurs in the ration or mineral supplement, the Ca : P ratio and the vitamin D content may greatly effect their absorption and utilization.

In order to throw further light on the calcium and phosphorus requirements of growing bovines, metabolism experiments over a long period, were undertaken. The animals were divided into different groups which were fed rations (*a*) supplemented with phosphates, (*b*) at different levels of mineral intake, and (*c*) at different Ca : P ratios.

II. LITERATURE.

A. Review Articles.

As a result of extensive research throughout the scientific world, there are now available voluminous data on the problems associated with mineral metabolism, and a detailed survey of the literature will not be undertaken here. Several exceedingly well compiled reviews have been published on calcium and phosphorus metabolism, a considerable portion of them being devoted to the deficiency diseases associated with these two elements.

Forbes and Keith (1914) published extensive work on the phosphorus compounds in animal nutrition. These authors described in detail the different forms in which phosphorus exists in organic and biological materials, the major portion of the work being devoted to the absorption, excretion, metabolism and requirements of phosphorus or its compounds in health and disease. It must however be pointed out that nearly all the work discussed therein belongs to the " pre-vitamin era ". Much of the work cited on laboratory animals is partly invalidated by that fact.

In his monograph " Minerals in Pasture " Orr (1929) gave valuable data with regard to the mineral content of pastures and pointed out the deficiencies, to which grazing animals are subject. In certain parts of South and East Africa, Australia, Norway and the United States of America the pastures were so deficient in calcium or phosphorus or both, that they were incapable of supporting normal growth of stock.

Crichton (1930) in a discussion of the mineral requirements of dairy cattle deplores the absence of information regarding the actual requirements of calcium and phosphorus for growing bovines. The author considered that the information relating to the subject was not immediately applicable in practice, because the work had been accomplished under conditions which had no parallel in practice.

A very extensive review of the factors associated with rickets in human beings and animals, was given by Goldblatt (1931). The subject was discussed from clinical, pathological, historical, radiological and chemical viewpoints, by far the greater portion of the work being devoted to the rôles played by calcium, phosphorus and vitamins in the etiology of rickets. The difference in chemical composition of the skeleton and body fluids of normal and rachitic animals and human beings was described, and the factors which affects the calcium and phosphorus utilisation in the ration, as well as the rôle of artificial and solar irradiation, causing normal or abnormal calcification, were discussed.

This review is probably the most complete published in recent years on rickets; over 2,700 references are quoted.

In their recent publications, Marek and Wellmann (1931, 1932) gave considerable data of their own experiments on dogs and pigs, as well as of the literature on calcium and phosphorus metabolism in a discussion of their conception of the causes of osteodystrophic diseases in animals. They argued that the mineral value of the ration is signified by the alkali-alkalizität, i.e. $(K_2O + Na_2O) - (Cl + SO_3)$ and erdalkali-alkalizität, i.e. $(CaO + MgO) - P_2O_5$ when these values are expressed in milligram equivalents per 100 gm. dry matter.

Sherman (1932, 1935) discussed in detail the mineral requirements of human beings, and laid great stress on the necessity of a calcium sufficient diet for health and growth. He pointed out that this most important element is usually deficient in the ordinary human diet.

In his review, Shohl (1933) aimed at bringing up to date from 1930, the literature associated with the many phases of calcium metabolism. Attention was given mainly to the forms of calcium in the blood, the metabolism of calcium and phosphorus and the factors influencing bone calcification. The author considered that magnesium metabolism is intimately related to that of calcium and phosphorus.

Theiler and Green (1932) discussed in detail the effect of a phosphorus deficiency in the feed of cattle and sheep grazing on natural pastures. The history, cause and effects of Lamsiekte in South Africa were given and compared with deficiency diseases in other parts of the world.

B. Calcium and Phosphorus Requirements.

Weiske (1873) concluded from metabolism experiments that 5 to 6 months old calves required 12·0 gm. Ca and 9·5 gm. P daily. The body of a calf was found to contain 1·2 per cent. Ca and ·67 per cent. P by Lawes and Gilbert (1883). From these analyses Armsby (1917) computed that for the first 12 months calves should daily retain 15·4 gm. Ca and 8·1 gm. P.

Kellner (1912) argued that a bovine weighing 350 Kgm. contained 1·57 per cent. Ca and ·88 per cent. P. If the constituents in the body at birth were disregarded, the animal would require 15 gm. Ca and 8·3 gm. P per day; the food should contain 2 to 3 times as much of each mineral, as only 33 to 50 per cent. of the minerals in the ration was utilised.

As shown by Starling (1920), there was a close relationship between the amounts of minerals present in the milk of the mother and the ash of the young for each species. From this Crichton (1930) argued, that for every 1 lb. of protein in cows milk, there was ·8 oz. CaO. According to Wolff-Lehmann's tables a bovine of 500 lb. required 1 lb. protein per day. It should, therefore, assimilate ·8 oz. CaO per day.

Wellmann (1932) considered that 100 Kgm. live weight required daily for maintenance 3·6 gm. Ca, about 2 gm. P, and 2·5 gm. P per 100 gm. gain in body weight. In the case of plant foods, three times as much calcium and twice as much phosphorus as that required, must be taken for production and growth. Archibald and Bennett (1935) expressed their results in a slightly different way. They found that dairy heifers grew normally during the first year on a ration which supplied 1·8 gm. P and 3·8 gm. Ca daily per 100 lb. live weight, the amounts decreasing as the animals grew older.

Theiler *et al* [1920, 1927 (*a*)] estimated that owing to the deficiency of phosphorus in the pasture the intake of ranching cattle in Bechuanaland during the dry season was only 6-11 gm. P_2O_5 per 1,000 lb. live weight. This dificiency of phosphorus caused severe osteophagia. They estimated that 27 gm. P_2O_5 per 1,000 lb. live weight was the minimum phosphorus requirement for the prevention of osteophagia in mature cattle. Of this 27 gm. P_2O_5, 20 gm. was supplied by a supplement of 3 oz. of bone meal.

These authors (1924) found that in other districts a supplement of ½-¾ lb. bonemeal per week was sufficient for the prevention of osteophagia in grazing animals, but expressed the opinion that this amount was probably insufficient for optimum growth and condition.

Theiler, Green and du Toit (1927) under stall-fed conditions produced "styfsiekte" in growing heifers by feeding a fanko hay ration containing 6·9 gm. CaO and 5·1 gm. P_2O_5. They expressed the opinion that this disease "styfsiekte" occurring naturally in South Africa, in cattle grazing on phosphorus deficient pastures, was probably identical with osteomalacia. Theiler later (1931) by making an extensive histological study of the bones of the animals used in the above experiment, showed that the condition of aphosphorosis known as Styfsiekte and produced artificially in growing heifers, was osteomalacia in the mature and was referred to as rickets in the growing animal.

These authors found that an intake of 4·8 gm. P was insufficient for growing heifers, although half of it was supplied in the form of bonemeal, actual signs of a deficiency not becoming evident until after calving. A supplement of 3 oz. of bonemeal bringing the

total daily intake of Ca to 37 gm. CaO and of P to 28 gm. P_2O_5 was found to supply the mineral needs of the heifers used in their experiment. Du Toit and Green (1930) concluded that a daily supplement of approximately 18 gm. P_2O_5 given in the form of bonemeal to growing bovines kept on phosphorus deficient pasture in the Bechuanaland area was probably in excess of their requirement for optimal growth, but that a daily supplement of 6 gm. P_2O_5 given as bonemeal was definitely below it .

Watkins (1933) found that a ration supplying 18 months old growing steers with 11·6 gm. Ca and 8·5 gm. P per day was sufficient with regard to these minerals. A slightly higher intake of phosphorus (10·8-12·3 gm.) was favoured by Lamb *et al* (1934) for growth and pregnancy in bovines from 18-30 months of age.

It would appear from the data quoted that young bovines will grow normally on a ration supplying about 20 gm. P_2O_5 and 20 gm. CaO per day. A considerable portion of this amount should however be in an available form such as in mineral supplements or in good quality hay.

C. Assimilation of Calcium and Phosphorus.

Maximum utilization of the food constituents is always aimed at in order to supply adequately the demands for growth, production and reproduction. Several factors impair the utilization of minerals even though the latter exist in an easily assimilable form.

The small intestine is the zone of absorption but the minerals absorbed are not necessarily retained, as a fair amount, especially calcium, is belived to be excreted into the large intestine, and to pass out with the faeces.

Meigs, Blatherwick and Cary (1919) expressed the opinion that the assimilation of calcium and phosphorus was facilitated by feeding these minerals alternatively, but no definite experiments have since been carried out to prove the validity of this theory.

McGowan (1933) maintained that for optimal utilization of Ca and P after absorption, it is essential that these minerals should be supplied in the food uncombined with one another and that they should be absorbed, as far as possible in this condition.

Intestinal absorption was proved by Miller, Yates and Jones (1926) to be partly controlled by the needs of the animal. In accordance with the above, Hodgson and Knott (1932) ascribed the poor utilization of minerals by dairy heifers, fed on artificially dried herbage, to the fact that when the animals were full-grown, their demands were very small.

Forbes *et al* (1922), Turner *et al* (1927, 1931), Haag *et al* (1929), Hayden, Monroe and Crawford (1930), Hart, Kline and Humphrey (1932) found negative mineral balances for high producing cows. In some of these cases more than 90 per cent. of the total mineral intake was excreted in the faeces and urine, the 10 per cent. left to supply the minerals contained in the milk being inadequate; the

animal was forced to draw on her skeletal reserves. Hart *et al* (1933) concluded that the animal was unable to increase its assimilation of minerals even when the mineral intake was considerable.

Mitchell *et al* (1931) found, with pregnant gilts, an average daily retention of 4·38 gm. Ca and 1·32 gm. P, being 31·5 per cent. and 16 per cent. of the intakes respectively.

Cows given 58 gm. Ca and 51 gm. P by Turner, Kane and Hale (1933) utilized about 31 per cent of each element. The ration consisted of Timothy hay, a grain mixture and a calcium supplement. Hart *et al* (1933) found with a high producing Ayrshire cow fed on a ration of alfalfa, grain and corn silage, a retention of almost 44 per cent. of the Ca intake.

According to Meigs *et al* (1934) cows kept for several months on an inadequate calcium ration, may utilize about 50 per cent. of the calcium content of the ration.

Forbes *et al* [1921 (*b*)] found that pigs fed on a grain ration containing a calcium salt retained 50-70 per cent. of the calcium, while Spiers and Sherman (1936) with rats and Morgen *et al* (1933) with dogs, found a retention of 90 to 93 per cent of the calcium intake. However as far as the author is aware, such high values have not been reported for sheep, pigs or cattle.

It must be pointed out, however, that that percentage utilization or retention means very little, as such factors as demand of the animal, the total intake, the form in which the minerals are given and the vitamin D content of the ration or solar irradiation, effect the retention. Obviously the percentage retention based on a high intake of Ca and P may be low and hence gives a poor idea of the utilization of these minerals. In the same way poorer utilization or a lower intake may show an appreciably higher percentage retention.

D.—Factors Effecting the Utilization of Calcium and Phosphorus.

Considerable work has been done to determine the relative availability of the calcium and phosphorus of different feeding stuffs and of mineral supplements, but the results obtained are by no means conclusive.

Kramer, Potter and Gillum (1930), Fincke and Sherman (1936) and Kohman and Sauborn (1935) with rats and Hart *et al* (1927, 1930, 1931) with bovines determined the availability of the calcium of various foods.

Hesse and Barndt (1933) concluded that the absorption of calcium from the calcium salts tested (viz. calcium carbonate, di-calcium phosphate, tri-calcium phosphate, calcium gluconate) is entirely independant of the solubility of these salts.

Attention has furthermore been given to the availability of the phosphorus in different phosphatic supplements, especially di-calcium phosphate and bonemeal. A better utilisation of calcium and

phosphorus was obtained by Bloom (1932) by feeding di-calcium phosphate than by feeding tri-calcium phosphate or bonemeal. Similarly, du Toit and Green (1930) concluded from supplementary feeding experiments with cattle, that taking weight for weight, di-calcium phosphate was three time more effective than bone meal. Di-calcium phosphate contains 42 per cent. P_2O_5 and bonemeal 24 per cent. P_2O_5. Later work by Malan and du Toit (1932) enabled them to place the supplements in the followign order of availability of the phosphorus.

(1) Sodium phosphate.

(2) Precipitated calcium phosphate.

(3) Bonemeal and degelatinised bone flour.

The conclusions of these authors were based on the weight increase of the animals.

The calcium and phosphorus of food such as cereals containing inositol phosphorus are poorly utilized. Booth, Henry and Kon (1935) and Bruce and Callow (1934) maintained that oatmeal contains mostly phytin phosphorus (inositol hexaphosphate) which they consider to be non-available to animals. According to Bleyer and Fisher (1931) and Forbes and Irving (1931) the Ca-Mg salt of inositol hexaphosphoric acid was well utilized by rats. The work of McCance and Widdowson (1935) also showed a partial utilization of the phytin ingested by human beings.

Steenbock *et al* (1930) observed that " equalization of the phosphorus content of cereal rations did not make them equally effective in bone formation ". No correlation was, however, found by Harris and Bunker (1935) between the phytin phosphorus content and the rachitogenic properties of the diet.

Later Templin and Steenbock (1933) found better calcification by feeding immature yellow dent field maize than when mature maize of the same variety and grown under identical conditions was fed. In a subsequent paper by these authors [1933 (*a*)] autolysed germinated maize was shown to possess definite antirachitic properties, when compared with mature maize.

Further work by Lowe and Steenbock (1936) clarified the position considerably. They showed that (a) the phytin phosphorus present in the rachitogenic diet was poorly available when compared with the phosphorus of phosphoric acid and sodium glycerophosphate and (*b*) treatment of maize with HCl improved its antirachitic properties in proportion to the extent that the phytin was hydrolyzed.

The anti-rachitic factor, Vitamin D, apparently regulates the absorption of calcium and phosphorus from the intestine and the deposition of minerals in the osseous tissue, to form bone.

A deficiency of vitamin D, affects the utilization of calcium and phosphorus detrimentally. Morgan Garrison and Hills (1933) maintained that vitamin D apparently prevents re-excretion of absorbed calcium into the intestine. The vitamin causes deposition of calcium to form bone rather than an increase of original absorption.

Rupel, Bohstedt and Hart (1933) and Bechel *et al* (1933) showed that calves require vitamin D in some form. Further work with calves by Wallis, Palmer and Gullickson (1935) and Duncan and Huffman (1936) indicated that the addition of vitamin D to a ration deficient in this constituent, but adequate in calcium and phosphorus, greatly increased the mineral retention. Negative mineral balances thereby became positive.

The animals used in the experiments reported in this publication were exposed to direct sunlight daily for several hours in their exercising paddocks. Hence abundant vitamin D was supplied in this way in addition to that contained in the feed.

It is generally accepted that the utilization of Ca and P is affected by the ratio in which these elements are present in the diet and from extensive investigations of this problem, it is generally conceded that a ratio of approximately 2 Ca to 1 P in the diet favours the best utilization of these elements. Theiler, Green and du Toit (1927) expressed the view that the current emphasis on Ca : P ratio was probably exaggerated especially when these elements were present in abundance in the ration of animals. At lower levels of intake the best utilization of these two minerals is an advantage to the animal and hence a correct ratio becomes more important; but here again it would seem that the level of intake of Ca or P or both becomes equally if not more important. For instance Brown, Shohl *et al* (1932) and Shohl (1932, 1936) stated that the Ca : P ratio inadequately defined the rachitogenic properties of a diet. Rickets could be produced in rats fed on diets low in phosphorus no matter what the ratio of Ca : P was. Theiler, du Toit and Malan (1937) have expressed the view that in the production of osteodystrophic diseases by limiting the intake of Ca or P or both, the ratio was decidedly of secondary importance. The rapidity with which the condition developed bore a direct relationship to the ratio, a normal ratio producing the condition by no means as rapidly as a wide ratio does, but the difference in the severity of the conditions produced ultimately not being always apparent.

It is doubted whether the ratio of dietary Ca to P is important to the health of animals under conditions of feeding which are usually considered normal except perhaps in the case of horses and pigs where the nature of the ration is such that it contains relatively high P and low Ca. Kintner and Holt (1932) believe that the ratio of 1 CaO to 2·5 P_2O_5 is dangerous to the health of horses and it is feared that this ratio is often present in their rations.. The wide ratio is effective in producing disease, but it must be remembered that the Ca is low in addition and it is freely admitted that the ratio will effect animals detrimentally under such conditions.

E.—Blood Analysis.

Robinson and Huffman (1926) found that the blood of normal mature cattle contained from 7·7 to 14·7 mg. of Ca per 100 ml. Allcroft and Green (1934) and Haag and Jones (1935), however, found values between the narrower limits of 8·6 to 11·6 mg Ca per

100 ml. about 90 per cent. of their values falling between 9 and 11 mg. It has been found by Kintner and Holt (1932) while investigating equine osteomalacia, Fitch *et al* (1933) and Groenewald (1935) with cattle, in experiments over a long period, Jones and Robson (1932) with rats and Fraser (1932) with sheep, that the serum calcium remained normal with diets deficient in calcium but with an adequate amount of vitamin D. When, however, a deficiency of both calcium and vitamin D existed in the ration, Auchinachie and Emslie (1932) and Fraser *et al* (1934) working with sheep, Bethke *et al* (1923) and Jones and Cohen (1936) with rats, found a lowered blood calcium. An addition of cod-liver oil (vitamin D) restored the serum calcium to the normal value.

With calves on an adequate mineral ration but lacking in vitamin D, Rupel, Bohstedt and Hart (1933), Huffman and Duncan [1935 (*a*)] found a lowering of the serum calcium, decreasing in some cases to 6 mgm. Ca per 100 ml. blood. The addition of cod-liver oil, however, promptly brought the concentration of this element in the blood back to normal.

Huffman and Robinson (1926), Palmer and Eckles (1927), Henderson and Weakly (1930), Malan, du Toit and Green (1928) showed that the phosphorus content of the ration of bovines had a marked influence on the inorganic phosphorus content of the blood. Such low values as 1·0 mgm. P per 100 ml. blood were obtained by Malan, du Toit and Green (1928) for mature cattle on phosphorus deficient grazing. Phosphorus sufficiency in the diet of mature bovines was reflected as an increase in the inorganic P content of the blood. Values above 3·5 mgm. per 100 ml. blood were regarded as normal for mature bovines.

Kramer and Holland (1923) with chickens and Rupel, Bohstedt and Hart (1933) with calves, showed that with an adequate mineral diet deficient in vitamin D the inorganic blood phosphorus dropped but was restored to normal when cod-liver oil was given.

The determination of inorganic phosphorus in the blood of animals provides an excellent way of diagnosing phosphorus deficiency in stock kept under conditions of vitamin D deficiency. In South Africa this method has been employed to collect information on the extent of phosphorus deficiency in stock kept on natural pastures.

According to Kay (1929-1930) a markedly raised concentration of plasma phosphatase in human bones was confined almost specifically to certain generalized diseases of the bone, increased values more than 20 times the normal being found.

Bodansky and Jaffe (1931, 1932) working with rats and guinea pigs found the plasma phosphatase to be affected by fasting and a deficient calcium intake.

A vitamin D deficiency, with adequate mineral intake, was found by Auchinachie and Emslie (1933) to increase the phosphatase content of the blood of sheep three to four times. These authors expressed the opinion that the concentration of plasma phosphatase gave much earlier indications of disordered calcium and phosphorus metabolism

than either serum calcium, blood inorganic phosphorus, general state of health or body weight. In agreement with the abovementioned workers, Bodansky and Jaffe (1934) considered plasma phosphatase values to be better criteria for the severity and progress of rickets than those of the serum calcium and phosphorus.

Totally unexpected plasma phosphatase values were found by Palmer *et al* (1935). The calcium intake, with or without vitamin D, had no effect on the plasma phosphatase of cows. The bone analysis indicated that there was no disturbed metabolism.

It is possible that the determination of plasma phosphatase is not equally valuable for diagnosing poor calcification or even abnormal Ca P metabolism in all species of animals.

F. Bone Analysis.

Apart from the primary functions of the skeleton to serve as a framework for the body, it plays a very important role, namely, as a reservoir for the important minerals calcium and phosphorus. From this mineral reservoir calcium and phosphorus are withdrawn during periods of mineral stress. The bone is resorbed as a whole decreasing in weight and density and increasing in porosity, and the bone becomes osteoporotic, unless complicated by osteomalacia, when uncalcified osteoid tissue is deposited. The result is a decreased ash content with or without an increase in oganic matter, thereby rendering the bones less rigid.

Histological examinations of bone sections give a clear picture of the condition of the animal with regard to calcium and phosphorus but as such a study falls outside the scope of this paper and is fully discussed by Theiler *et al* (1937), only the physical and chemical analysis of bone will be discussed.

Forbes and his co-workers [1921 (*a*), (*b*), (*c*), (*d*)] made an extensive study of the causes of bone fragility in brood sows. Marked differences were shown in the different groups for the ash per c.c. bone (volume of bone taken), the breaking strengths of the bones and the live weight of the animals.

Rottensten and Maynard (1934) found that the average dry-fat-free femurs of rats on a low level of mineral intake, contained 59·6 per cent ash as against 64 per cent for the bones of animals receiving an adequate mineral supply. The average ash content of the dry-fat-free cannon bones of calves on a concentrated ration, was found to be 56·8 per cent by Mead and Regan (1931). The addition of calcium to this ration brought the ash content for the same bones of other calves to 64·5 per cent. Similar figures are given by Kintner and Holt (1932) for the metacarpal ash content of horses.

Bethke, Edgington and Kick (1933), Rupel, Bohstedt and Hart (1933) and Shohl (1936) concluded that the ash content of bone affords an excellent index of the degree of calcification. The figures for the breaking strength of bones by Becker, Neal and Shealy (1933-1934) were striking. Heavy producing cows were kept for several lactation periods on calcium deficient rations. Cow No. 59,

after eleven consecutive lactations, fractured her pelvic arch in three places. The average breaking strength of her femurs and humeri over a six inch span, was 335 lb. per square inch as against over 3000 lb. per square inch for the same bones of animals receiving supplements of bone meal.

Hogan (1932) and Aubel, Hughes and Lienhardt (1936) found with pigs that the level of mineral intake had hardly any effect upon the diameter of the bone, but remarkably influenced the thickness of its wall.

Blum (1931), Holmes and Pigott (1931) and Hartman and Meigs (1931) showed that the Ca : P ratio of the bones of animals can not be altered by varying the mineral content of the ration. In contradiction with the above, Yeager and Winters (1935) concluded that there was some indication that the Ca : P ratio in the bones depended to some extent at least on the amounts of calcium and phosphorus present in the diet. This ratio, with animals fed at varying levels of mineral intake, was found by Brooke, Smith and Smith (1934) to vary from 2·3 : 1·0 to 2·1 : 1·0.

III. GENERAL DESCRIPTION OF INVESTIGATION.

A. Object.

The following investigations were undertaken with the object of studying—

(1) the availability of the phosphorus contained in different commercial phosphates which are used extensively as stock feed in the Union of South Africa;

(2) the effect of different levels of mineral intake upon the utilization of the calcium and phosphorus, when growing bovines are fed the same basal ration with a constant calcium to phosphorus ratio of 2 : 1;

(3) the effect of varying the calcium to phosphorus ratio of the ration upon the utilization of these minerals.

In the experiment to be reported on, the retention of these minerals was studied on young bovines growing normally and on those reared under adverse conditions which are often encountered in practical stock farming in the Union of South Africa.

B. Procedure.

The animals used for these experiments were young bovines which were periodically placed in concrete floored stalls, so constructed that the urine could be run off into receptacles sunk into the floor immediately after being voided. The faeces were collected by an attendant who was on duty day and night. As a result of this arrangement no intermixing of faeces and urine took place. The stalls were large enough to allow the animals to move freely and to lie down whenever they wished.

All the urine and the faeces voided by an animal during each week were gathered separately. To each collection formalin was added in order to avoid putrefaction and to render the samples less objectionable to work with. The moisture content of the faeces was determined while Ca and P were determined on both urine and faeces.

The basal ration contained a sufficient quantity of poor quality hay to provide roughage. The ingestion of calcium and phosphorus was thus kept as low as possible. Fanko (rolled maize endosperm) or samp (unrolled maize endosperm) both of which are very low in minerals, comprised the major portion of the ration and provided most of the energy and some of the protein. Meat meal or blood meal was added to increase the quantity and quality of the proteins, green feed or silage being included to avoid a possible deficiency of vitamins A and C. Full particulars of the animals and the rations will be given at the commencement of each experiment.

Twice daily each animal was fed a weighed quantity of feed. Tap water, of known calcium content, was provided, the total imbibed by each animal measured and recorded during the periods when faeces and urine were collected. Prior to the feeding of the experimental ration, the animals were kept in the stalls and fed the basal ration in order to accustom them to the conditions under which the experiment was to be conducted. If the animal did not finish the feed supplied, the remaining portion was weighed and recorded. An endeavour was subsequently made to regulate the quantity supplied in such a manner that nothing or very little remained over. When food was left over by an animal during a metabolism trial, its calcium and its phosphorus contents were determined and deducted from the intake.

The mineral content of each constituent of the basal ration was determined from time to time. This procedure was deemed necessary since considerable variations were obtained in the mineral content of different consignments of food.

The minerals intended for each animal were weighed out and added to the rations as required.

During the periods of rest when faeces and urine were not collected, the animals were allowed to exercise in a paddock during the day, where they had free access to water and sunlight. The paddock had a concrete floor to prevent earth eating.

The animals were weighed and bled monthly, these operations being carried out early in the morning before feeding and watering.

C. Methods of Analysis.

The technique adopted was that outlined by Malan and van der Lingen (1931) for taking blood samples and for determining inorganic blood phosphorus and blood calcium.

The calcium and phosphorus contents of the feeds and the excreta were determined by the method given by the same authors for the analysis of grasses. Benedict and Theis (1924), Fiske and Subbarrow (1925) and Roe and Kahn (1926-1929) have contributed to the

standardization of these methods. A modification of the method of Clark and Collip (1925) for the determination of calcium in the feeds and excreta was introduced since it was found to be more applicable. The calcium and the phosphorus contents of the bone ash were determined by the standard macro-volumetric methods described by Treadwell and Hall (1930).

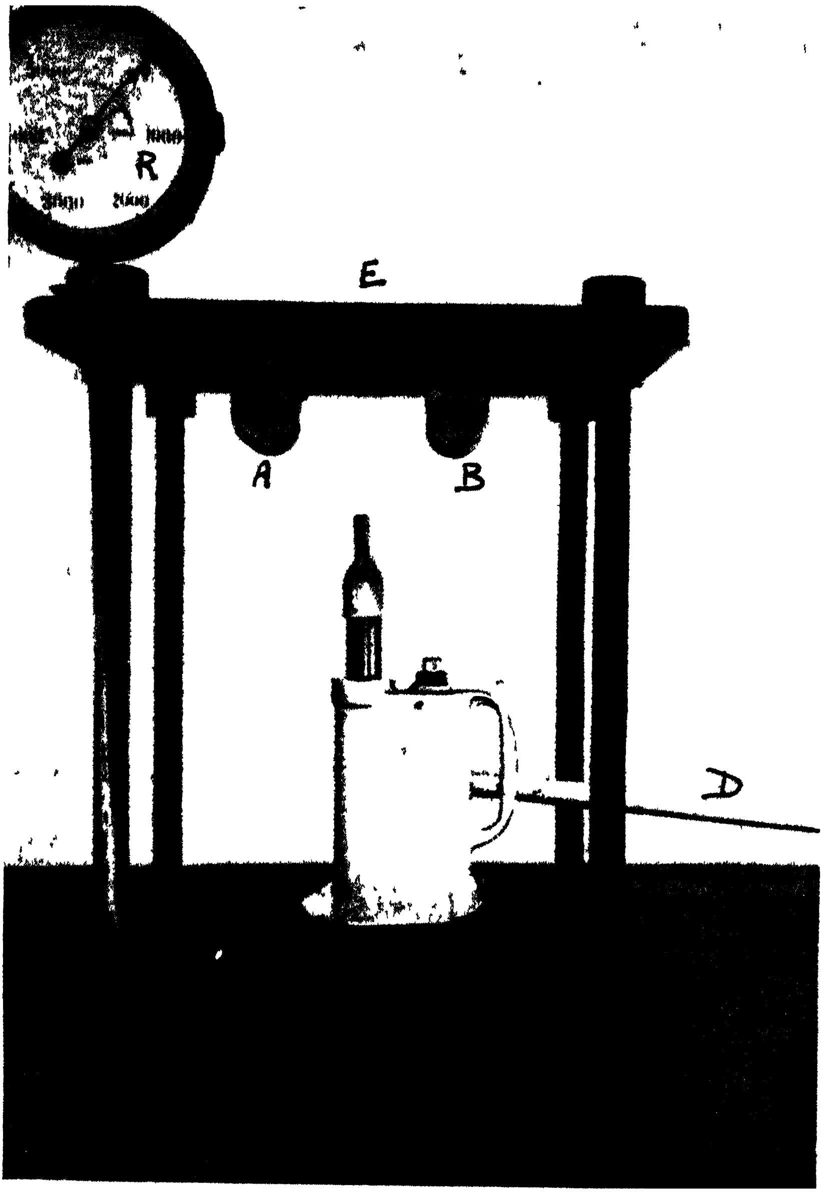

Fig. 1.

A and B are two adjustable rollers which can be set at any required distance apart. AE must always be equal to EB.

The animals were destroyed at the end of each experiment for the purpose of collecting the bones for chemical and histological examinations. All the meat was quickly and carefully removed from the bones without undue exposure of the latter to prevent loss of moisture.

Immediately after the bone had been cleaned, it was weighed and its measurements taken. The volume was obtained by immersion into water contained in graduated glass cylinders or specially marked glass jars, the vessels used depending upon the size of the bones. After removal from the water, the bone was dried by rubbing with a cloth. The breaking strength was then determined over a six inch span. The apparatus used for this purpose was a small scale model of a steel testing machine (Fig. 1).

Fig. 2.

In order to determine the breaking strength of the bone its centre is placed on the point C and by applying pressure by means of the lever D the bone is forced against the points A and B. The breaking pressure of the bone is registered on the pressure gauge R as pounds per square inch. The position in which the different bones were placed is indicated in Fig. 2.

The bone was then broken up with the aid of a chissel and hammer into small pieces about an inch long.

These fragments of bone and marrow were then collected in a receptacle and placed in a hot air oven at 105° C. for 48 hours in order to determine the water content by loss of weight. The fat was poured off and the pieces of bone collected in a linen bag and placed in a fat extractor, where the remaining fat was extracted with ether. After removal from the extractor the bones were dried in a hot air oven in order to obtain the dry-fat-free weight. The pieces of bone were then reduced to bonemeal in a bone shredder and an electric mill for the determination of ash, calcium and phosphorus.

IV. EXPERIMENT I.

The Availability of the Phosphorus contained in Different Phosphatic Supplements.

Extensive feeding of phosphorus to stock has resulted in the marketing of a number of phosphatic products. Bonemeal has been greatly favoured by stock farmers as a supplementary feed, it being the first to be recommended and easily procurable. The different brands of this product vary in colour from a dirty brown to a clear creamy white, depending on the treatment during the process of manufacture. For this reason it was considered advisable to investigate the assimilability of the phosphorus contained in the various bone meals as well as that of other phosphatic products commonly used.

The commercial phosphatic supplements generally used may be classed under three main types. Di-sodium phosphate and mono-ammonium phosphate were used as examples of water soluble phosphates which can be administered in solution in the drinking water of stock. Precipitated calcium phosphate which is mainly di-calcium phosphate is also extensively used. It is prepared as a by-product in the manufacture of gelatine from bones or is obtained from rock phosphate. This product is probably the cheapest for the supplementary feeding of stock. Bonemeal is used more extensively than any other phosphate and was therefore included in the experiment.

Experiment I (a).

Two half grade Friesland bovines were used in this experiment. At the commencement of the work their ages and weights were as follows:—

No.	*Age.*	*Initial weight.*
5151	15 months..................	490 lb.
5164	17 months..................	630 lb.

The basal ration with its calcium and phosphorus contents is tabulated below.

Feed.	Amount.	% P.	P gm.	% Ca.	Ca gm.
Hay........................	1·32 Kg.	0·048	1·634	0·1320	1·742
Fanko......................	3·00 Kg.	0·0393	1·180	0·0071	0·213
Meatmeal..................	·32 Kg.	0·247	0·560	0·043	0·099
Water......................	± 10 litres	—	—	-	0·583
Sodium chloride............	15 gm.	—	—	—	—
Total intake................	-	—	2·38 gm. P.	—	2·64 gm. Ca.

The mineral intake varied slightly with the mineral contents of the ration and the water supplied. Analyses of these were made form time to time.

TABLE I.

Time-table for the Feeding of Supplements and the Collection of Faeces and Urine.

Week No.		Supplement.	Remarks.
1		0	Rest.
2		0	Collection of excreta.
3	A.	Di-sodium phosphate + Calcium Carbonate....	Rest.
4	A.	3·5 gm. P + 9·6 gm. Ca..................	Collection.
5	A.	3·5 gm. P + 9·6 gm. Ca....	Collection.
6		0	Rest.
7		0	Collection.
8	B.	V. Bonemeal + calcium carbonate............	Rest.
9	B.	3·5 gm. P + 10 gm. Ca....................	Collection.
10	B.	3·5 gm. P + 10 gm. Ca....................	Collection.
11		0	Rest.
12		0	Collection.
13		0 (change of hay).. ..	Rest.
14		0	Collection.
15	C.	H. Bonemeal + calcium carbonate............	Rest.
16	C.	2·79 gm. P + 7·42 gm. Ca................	Collection.
17	C.	2·79 gm. P + 7·42 gm. Ca................	Collection.
18		0	Rest.
19		0	Collection.
20	D.	Mono-ammonium phosphate + Calcium carbonate	Rest.
21	D.	2·88 gm. P + 7·42 gm. Ca................	Collection.
22	D.	2·88 gm. P + 7·42 gm. Ca................	Collection.
23		0	Rest.
24		0	Collection.
25	E.	Di-calcium phosphate + Calcium carbonate....	Rest.
26	E.	2·97 gm. P + 7·57 gm. Ca................	Collection.
27	E.	2·97 gm. P + 7·57 gm. Ca................	Collection.
28		0	Rest.
29		0	Collection.

The supplements tested out in this experiment were (A) Di-sodium phosphate, (B) V. Bonemeal, (C) H. Bonemeal, (D) Mono-ammonium phosphate and (E) Di-calcium phosphate.

Calcium carbonate was added to each supplement in order to keep the calcium-phosphorus ratio of the mineral intake constant.

Both animals were treated in exactly the same manner. They were fed on the same ration and supplements at the same time according to Table I.

The results of the balance trials are given in Tables II to III.

TABLE II.

Mineral Balance (daily average).

Supplement A = Na_2HPO_4 + $CaCO_3$ i.e. 3·5 gm. P + 9·6 gm. Ca.

B = V. Bonemeal + $CaCO_3$ i.e. 3·5 gm. P + 10 gm. Ca.

C = H. Bonemeal + $CaCO_3$ i.e. 2·79 gm. P + 7·4 gm. Ca.

D = Mono-ammon. phosphate + $CaCO_3$ i.e. 2·88 gm. P + 7·42 gm. Ca.

E = Di-calcium phosphate + $CaCO_3$ i.e. 2·97 gm. P + 7·75 gm. Ca.

Bovine 5151.

Week No.	Supplement.	Intake.		Outgo gm. P.		Outgo gm. Ca.		Balance.	
		gm. P.	gm. Ca.	Faeces.	Urine.	Faeces.	Urine.	gm. P.	gm. Ca.
1	O	2·38	2·63	2·81	0·31	4·71	1·74	–0·74	–3·28
2	O	2·38	2·65	3·26	0·24	4·81	1·79	–1·12	–3·95
3	A	5·88	12·23	2·98	0·23	6·53	0·51	2·67	5·19
4	A	5·88	12·26	3·02	0·21	7·85	0·47	2·65	3·94
5	O	2·38	2·61	3·39	0·15	3·64	1·51	–1·16	–2·54
6	B	5·93	12·66	3·59	0·25	8·03	0·54	2·09	4·09
7	B	5·93	12·66	3·33	0·19	8·18	0·46	2·41	4·02
8	O	2·77	2·70	3·05	0·25	4·41	1·36	–0·53	–3·07
9	O	3·67	4·42	3·23	0·23	4·20	1·47	0·21	–1·25
10	C	6·46	11·92	3·46	0·21	6·51	0·45	2·79	4·96
11	C	6·46	11·95	3·58	0·20	5·96	0·45	2·68	5·54
12	O	3·67	4·49	2·98	0·22	3·86	1·66	0·47	–1·03
13	D	6·55	11·85	3·15	0·24	4·96	0·44	3·16	6·45
14	D	6·55	11·83	3·29	0·31	5·58	0·41	2·95	5·84
15	O	3·67	4·42	3·43	0·23	4·46	1·84	0·01	–1·88
16	E	6·64	11·99	3·45	0·27	5·93	0·46	2·92	5·60
17	E	6·64	11·99	3·75	0·30	5·88	0·41	2·59	5·70
18	O	3·67	4·42	2·91	0·23	3·64	1·74	0·53	–0·96

TABLE III.

Mineral Balance (daily average).

Supplement A = Na_2HPO_4 + $CaCO_3$ i.e. 3·5 gm. P + 9·6 gm Ca.
B = V. Bonemeal + $CaCO_3$ i.e. 3·5 gm. P + 10 gm. Ca.
C = H. Bonemeal + $CaCO_3$ i.e. 2·79 gm. P + 7·4 gm. Ca.
D = Mono-ammon. phosphate + $CaCO_3$ i.e. 2·88 gm. P + 7·42 gm. Ca.
E = Di-calcium phosphate + $CaCO_3$ i.e. 2·97 gm. P + 7·5 gm. Ca.

Bovine 5164.

Week No.	Supplement.	Intake.		Outgo gm. P.		Outgo gm. Ca.		Balance.	
		gm. P.	gm. Ca.	Faeces.	Urine.	Faeces.	Urine.	gm. P.	gm. Ca.
1	O	2·41	2·63	3·10	0·24	4·98	2·07	−0·93	−4·42
2	O	2·41	2·59	3·23	0·25	4·88	1·95	−1·07	−4·16
3	A	5·91	12·23	3·33	0·19	5·83	0·61	2·39	5·78
4	A	5·91	12·22	3·53	0·17	7·51	0·30	2·21	4·41
5	O	2·41	2·61	3·22	0·17	2·77	1·22	−0·98	−1·35
6	B	5·96	12·66	3·76	0·19	7·23	0·39	2·01	5·04
7	B	5·96	12·64	3·87	0·19	8·09	0·30	1·90	4·25
8	O	2·80	2·70	3·37	0·22	4·18	1·06	−0·79	−2·54
9	O	3·74	4·42	2·89	0·21	4·71	2·08	0·64	−2·3[illegible]
10	C	6·53	11·92	3·40	0·23	5·38	0·41	2·90	6·13
11	C	6·53	11·90	3·65	0·24	6·35	0·44	2·64	5·11
12	O	3·74	4·49	3·20	0·23	3·94	1·74	0·31	−1·19
13	D	6·62	11·85	4·06	0·24	6·10	0·43	2·32	5·32
14	D	6·62	11·85	3·36	0·24	5·62	0·43	3·02	5·80
15	O	3·74	4·40	3·29	0·23	4·36	1·82	0·22	−2·74
16	E	6·71	11·90	3·26	0·26	6·28	0·56	3·19	5·15
17	E	6·71	11·98	3·72	0·26	6·05	0·53	2·63	5·40
18	O	3·74	4·42	3·00	0·20	3·73	1·53	0·54	−0·84

Discussion.

Some difficulty was experienced in determining a satisfactory basis for comparing the availability of the phosphorus contained in the different phosphates.

Although the minerals of a compound have to be made available during digestion, i.e. be present in a form that would pass through the intestinal wall, in order to be retained, they may nevertheless not be retained although highly available, as in the case of an animal in equilibrium on a high level of mineral intake. In such a case it cannot be assumed that the Ca and P are not absorbed, as the minerals absorbed in any one part, do not show in the balance experiment, although they have been in circulation. There is no method of distinguishing, in the faeces between the amount of minerals not absorbed and that re-excreted by the intestines after absorption.

In this study the proportion of Ca and P retained has been accepted as the measure of the availability of the product in which the calcium and phosphorus were present. Such a procedure is justified on the basis that the Ca and P contained in the supplements fed were well below the requirement of the animals. In other words, conditions were created for the retention of as large a proportion

as possible of the minerals fed, which obviously could serve as an indication or even measure of the availability of the Ca and P present in the products given.

When the animals were fed the basal ration, they showed negative mineral balances. (The phosphate balances became slightly positive when the mineral content of the basal ration increased.)

The daily basal ration contained 2·38 gm. P and was increased to 5·88 during the third and fourth week by the addition of 3·5 gm. P_2O_5 as di-sodium phosphate. The negative P balance became definitely positive, 2·66 gm. P being retained. If the assumption is made that no more P of the basal ration was retained during the period of feeding Na_2HPO_4 than when only the basal ration was given it is at once evident that an astonishingly large proportion of the P of the supplement was retained, viz., $\frac{2\cdot 66 + \cdot 93^{(1)}}{3\cdot 5} \times 100 = 108\cdot 6$ per cent. in the case of animal No. 5151 and $\frac{2\cdot 3 + 1}{3\cdot 5} \times 100 = 94\cdot 6$ per cent in cases of No. 5164. It would seem, therefore, that during the period of feeding Na_2HPO_4 either (1) some of the P of the basal ration was retained and that a balance on the basal ration, during the period immediately before the supplement was given, is not always a correct indication of the availability of P in the basal ration during a subsequent period of P feeding or (2) that all the P of the water soluble supplement fed under the conditions of the experiment was retained, in which case the P of the supplement should be regarded as 100 per cent. available.

This argument applies in a general way during the periods when the first two supplements were being fed. After that period the P balances on the basal ration were decidedly less negative or even slightly positive suggesting some utilization of the P of the basal ration or at least that the P present in the basal ration was sufficient to keep the animals in equilibrium with regard to P.

It must be noted that the increase in the amount of basal ration fed, resulted in an increase of P ingested from 2·41 to 3·74 gm. This quantity was apparently sufficient to keep the animals in equilibrium with regard to P as reference to the tables indicates.

Incidentally it may be mentioned that the total retention of P during the periods of supplying the phosphate supplement, shows little difference in spite of a smaller quantity of P being supplied in the supplement, a fact which again suggests 100 per cent. utilization of the supplementary P or increased utilization of the P present in the basal ration compared with that during the period of no supplementary feeding.

In regard to the retention of Ca as presented in Tables 2 and 3 it is surprising that the negative calcium balances were so low when the basal ration was the only source of Ca. Furthermore, relatively large quantities of Ca were retained during the period of supplementary feeding. So much so that it appears practically certain that

(1) (Average daily balance for 2 weeks of supplementary feeding) — (Average for week(s) prior to and week after supplementary feeding)

some Ca of the basal ration was utilized while the supplement was given, unless one assumes 100 per cent. utilization of the supplementary Ca. For instance in case of animal No. 5164 during the 10th and 11th trial the percentage retention of supplementary Ca, if only this fraction could be retained was $\frac{5 \cdot 45 + 1 \cdot 78}{7 \cdot 42} \times 100 = 97 \cdot 5$. This is an unlikely result and suggests that the Ca balance during the feeding of the basal ration only, did not necessarily apply to the period of supplementary feeding. Nevertheless, in order to arrive at a basis of comparing the percentage retentions on the availabilities of P and Ca in the different supplements, it is assumed that the P and Ca balances during the periods when only the basal ration is fed do apply during the period immediately following that when the supplement is fed.

For six weeks prior to the commencement of the experiment the animals received a ration very low in calcium and phosphorus. Weeks 1 and 2 reported in the balance tables, were carried out during the 7th and 8th weeks respectively during which the mineral deficient ration was fed; a depletion of minerals from the skeleton was shown.

Young growing bovines approximately 16 months old whose skeletons required considerable amounts of minerals to grow to maturity were used. The total mineral intake did not exceed the amount considered to be the optimal requirement for this type of animal but was well below it. The supplement was only fed for 3 out of 5 weeks, the animals being on a very low mineral intake for the remaining two weeks; thus reducing the average intake considerably. Furthermore the fact that such high retention figures were actually recorded, show that the positive balances probably do not represent the maximum the animal was capable of storing at the time.

TABLE IV.

Supplementary Phosphorus Retained.

	*	A.	B.	C.	D.	E.	*Mean.*
5151	1st week	1·089	0·840	0·878	1·014	0·892	0·963
	2nd week	*1·083*	*0·931*	*0·839*	*0·941*	*0·781*	*0·915*
	Mean	1·086	0·886	0·859	0·978	0·837	0·929
5164	1st week	0·971	0·829	0·867	0·712	0·946	0·865
	2nd week	*0·920*	*0·797*	*0·774*	*0·955*	*0·758*	*0·841*
	Mean	0·946	0·813	0·821	0·834	0·852	0·853
Mean	1st week	1·030	0·835	0·873	0·863	0·919	0·904
	2nd week	*1·002*	*0·864*	*0·807*	*0·948*	*0·770*	*0·878*
	Mean	1·016	0·849	0·840	0·906	0·844	0·891

* The 1st and 2nd week denotes the 2nd and 3rd week respectively of supplementary feeding, i.e. the two weeks during which a mineral balance was determined on the supplemented ration.

The basal ration of the two animals was supplemented successively with five different compounds of phosphorus. As previously stated, each supplement was given continuously for three weeks, during two of which the determinations were made. Between the different periods of supplementary feeding periods of no-supplement existed.

It is possible, from the data collected, to determine the difference in the retention of the phosphorus between—

(a) the different supplements;

(b) the two animals;

(c) the two weeks (i.e. after the animals had received the supplement for one week or for two weeks respectively).

It was found from an analysis of the animal variance that there was only an indication of a slight variation in the respective retentions of phosphorus by the two animals, an indication which is not borne out by a difference in the retention of Ca between the two animals. The conclusion is not justified therefore, that any real difference existed between the two animals with regard to their respective retentions of Ca and P.

In order to study the relative retentions of phosphorus supplied by the different supplements, the following table must be referred to:

TABLE IV (*a*).

Mean Proportion of Phosphorus retained.

A. *Di-Sod. phosphate.*	B. *V. Bonemeal.*	C. *H. Bonemeal.*	D. *Mono-Am. Phosphate.*	E. *Di-Cal. phosphate.*
1·016	0·849	0·840	0·900	0·844
Mean percentage of phosphorus retained.				
101·6	84·9	84·0	90·6	84·4

When the differences between the two means were compared by the t-tests (Fisher, 1935), it was found that A appeared to be greater than B, C and E, while all the other differences were insignificant. The relative decreasing order was :—

A, D, B, E and C.

The means for the two animals agreed very closely, being 0·878 for bovine 5164.

From Table IV (*a*) it will be seen that the phosphorus retention figures are exceedingly high, ranging from 100 per cent. for bonemeal and dicalcium phosphate. These high retention figures indicate that the optimal phosphorus requirement of the animals was not exceeded when these supplements were fed. The retention figures could therefore be used as a measure of the availability of the phosphorus in these supplements. The phosphorus of sodium phosphate may be considered as totally available whereas that of mono-ammonium phosphate, the two bonemeals and di-calcium phosphate is from 90-84 per cent.

It is remarkable that such high proportions of the phosphorus in the phosphatic supplements fed, were retained by the animals, as a matter of fact the differences in P retention among the supplements are insignificant compared with the high values obtained for the retentions in the case of each supplement. This observation is of great practical significance because it suggests that utilized P

supplements when given below optimal quantities are significantly better than is generally accepted. In the experiment under discussion, it would have been interesting to compare the values obtained with those at higher levels of intake of P.

The retentions of Ca are compared in table V below.

TABLE V.

Supplementary Calcium retained.

		A.	B.	C.	D.	E.	*Mean.*
B. 5151..	1st week...........	0·879	0·691	0·822	1·066	0·927	0·877
	2nd week..........	*0·749*	*0·684*	*0·898*	*0·984*	*0·941*	*0·851*
	Mean..............	0·814	0·688	0·860	1·025	0·934	0·864
B. 5164..	1st week...........	0·890	0·699	1·066	0·982	0·914	0·910
	2nd week..........	*0·747*	*0·620*	*0·929*	*1·047*	*0·947*	*0·858*
	Mean..............	0·819	0·660	0·998	1·015	0·931	0·884
Mean.....	1st week...........	0·885	0·695	0·944	1·024	0·921	0·894
	2nd week..........	*0·748*	*0·652*	*0·914*	*1·016*	*0·944*	*0·855*
	Mean..............	0·816	0·674	0·939	1·020	0·932	0·875

TABLE V (*a*).

Mean Proportion of Calcium retained.

A.	B.	C.	D.	E.
Di-Sod. phosphate.	*B. Bonemeal.*	*H. Bonemeal.*	*Mono-Ammon. Phosphate.*	*Di-Calcium phosphate.*
0·816	–0·674	0·939	1·020	0·932
		Mean percentage of Calcium retained.		
81·6	67·4	93·9	102·0	93·2

When the separate means for the different forms of supplement are compared (by the t-tests referred to above) the retention of calcium for the mono-ammonium phosphate, di-calcium phosphate and H. Bonemeal periods was significantly greater than for that of the di-sodium phosphate and V. Bonemeal periods.

The calcium retention when the different phosphate supplements (plus calcium carbonate, where necessary) were fed, varied from 100 per cent. for mono-ammonium phosphate to 67·4 per cent. for V. Bonemeal.

There is no correlation between the amount of phosphorus and the amount of calcium retained from period to period but the proportion of Ca retained was remarkably high in all the animals.

Experiment I (b).

In view of the results of the preceeding experiment it was decided to conduct similar investigations with two other animals in a similar manner. One from each of the three main types of phosphates, namely (A) di-sodium phosphate, (B) di-calcium phosphate and (C) bonemeal was selected for the purpose of the test.

Animals.

Two Young Half-grade Herefords.

No.	*Initial Age.*	*Initial Weight.*
5429	12 Months....................................	510 lb.
5436	12 Months....................................	475 lb.

Ration.

Feed.	Amount.	Percentage P.	P gm.	Percentage Ca.	Ca gm.
Samp.........................	1·50 Kg.	·037	·56	·015	·23
Hay..........................	·25 Kg.	·039	·10	·240	·60
Bloodmeal....................	·10 Kg.	·109	·11	·136	·14
Greenfeed....................	·50 Kg.	·047	·24	·128	·64
Salt Mixture A..............	25 gm.	—	—	—	—
Water........................	+ 10 litres	—	—	—	·53
TOTAL INTAKE....			1·01		2·14

The quantities given above were fed at the commencement of the experiment but were increased from time to time until finally the animals received the following ration.

3·0 Kg. Samp.
·5 Kg. Hay.
·5 Kg. Green feed.
·2 Kg. Blood meal.
25 gm. Salt mixture A.*

The final ration contained about 2·0 gm. P and about 2·8 gm. Ca.

The animals were kept for five weeks on the basal ration before faeces and urine were collected to determine the mineral balance on the basal ration.

The experimental animals were kept under the same conditions as those already discussed while the same general routine and technique applied. Each supplement was fed on three occasions in the course of the experiment.

The result of the mineral balances are given in Tables VI and VII.

* NOTE.—Salt mixture A: $NaHCO_3$ — 100 gm., $NaCl$ — 60 gm., K_2SO_4 — 60 gm., $FeSO_4$ — 2·5 gm., $Mg(OH)_2$ — 2·75 gm.

Table VI.

Mineral Balance (daily average).

Bovine 5429.

Supplement A = Na_2HPO_4 + $CaCO_3$ i.e. 4·58 gm. P + 11·27 gm. Ca.
B = Di-calcium phos. + $CaCO_3$ i.e. 4·37 gm. P + 11·27 gm. Ca.
C = V. Bonemeal + $CaCO_3$ i.e. 4·59 gm. P + 11·28 gm. Ca.

Week No.	Supplement.	Intake.		Outgo gm. P.		Outgo gm. Ca.		Balance.	
		gm. P.	gm. Ca.	Faeces.	Urine.	Faeces.	Urine.	gm. P.	gm. Ca.
Trial I—									
1	O	1·01	2·17	1·49	0·03	2·35	1·27	−0·51	−1·45
2	O	1·01	2·14	1·29	0·04	2·73	1·47	−0·32	−2·06
3	A	5·59	13·40	1·75	0·06	6·62	0·68	3·78	6·09
4	A	5·59	13·38	1·32	0·07	10·22	0·68	4·20	2·48
5	O	1·01	2·21	1·64	0·05	3·34	1·18	−0·68	−2·31
6	O	1·29	2·66	1·49	0·05	3·59	1·52	−0·25	−2·45
7	B	5·66	13·86	1·69	0·04	9·53	0·65	3·93	3·68
8	B	5·66	13·87	2·05	0·04	9·34	0·54	3·57	3·99
9	O	1·29	2·66	2·39	0·08	2·68	1·44	−1·18	−1·46
10	C	5·88	13·94	2·54	0·08	8·50	0·55	3·26	4·89
11	C	5·88	13·95	3·40	0·05	8·99	0·66	2·43	4·30
12	O	1·29	2·64	2·33	0·05	2·73	1·27	−1·09	−1·36
Trial II—									
13	O	1·71	2·64	2·17	0·03	2·79	1·80	−0·49	−1·95
14	A	6·27	13·96	2·23	0·09	5·11	0·71	3·95	8·14
15	A	6·27	13·90	2·44	0·08	5·55	0·77	3·74	7·58
16	O	1·71	2·66	2·01	0·05	2·03	2·24	−0·35	−1·61
17	B	5·94	13·45	1·89	0·03	5·38	0·64	4·02	7·43
18	B	5·94	13·43	2·13	0·05	7·66	0·57	3·76	5·20
19	O	1·71	2·67	2·13	0·04	2·48	1·47	−0·46	−1·28
20	C	6·24	13·44	1·89	0·05	5·28	0·73	4·30	7·43
21	C	6·24	13·43	2·23	0·04	7·69	0·63	3·97	5·11
22	O	1·71	2·66	2·36	0·05	1·80	1·38	−0·68	−0·52
Trial III—									
23	O	1·71	2·63	2·55	0·04	1·91	1·44	−0·88	−0·72
24	A	6·26	13·37	2·15	0·03	4·28	0·34	4·08	8·75
25	A	6·26	13·36	2·26	0·04	6·30	0·64	3·96	6·42
26	O	1·65	2·66	2·59	0·04	4·28	1·22	−0·98	−2·84
27	B	5·86	13·47	2·98	0·04	7·66	0·69	2·84	5·12
28	B	5·86	13·49	3·06	0·04	6·55	0·74	2·76	6·20
29	O	1·65	2·64	2·37	0·07	3·14	1·16	−0·79	−1·66
30	C	6·13	13·44	2·42	0·08	6·15	0·93	3·63	6·36
31	C	6·13	13·40	2·79	0·05	7·28	0·66	3·29	5·46
32	O	1·65	2·63	2·56	0·04	3·09	1·97	−0·95	−2·43

Table VII.

Mineral Balance (daily average).

Bovine 5436.

Supplement A = Na_2HPO_4 + $CaCO_3$ i.e. 4·58 gm. P + 11·27 gm. Ca.
B = Di-calcium phos. + $CaCO_3$ i.e. 4·37 gm. P + 11·27 gm. Ca.
C = V. Bonemeal + $CaCO_3$ i.e. 4·5 gm. P + 11·28 gm. Ca.

Trial I—

Week No.	Supplement.	Intake.		Outgo gm. P.		Outgo gm. Ca.		Balance.	
		gm. P.	gm. Ca.	Faeces.	Urine.	Faeces.	Urine.	gm. P.	gm. Ca.
1	O	1·01	2·14	1·42	0·03	2·11	1·31	−0·44	−0·28
2	O	1·01	2·15	1·53	0·05	2·26	1·11	−0·57	−1·22
3	A	5·59	13·46	1·26	0·07	7·50	0·50	4·26	5·46
4	A	5·59	13·45	1·39	0·07	8·36	0·89	4·13	4·30
5	O	1·01	2·19	1·64	0·03	2·48	1·78	−0·66	−2·07
6	O	1·29	2·64	2·12	0·05	3·03	1·52	−0·88	−1·91
7	B	5·66	13·86	2·37	0·05	8·18	0·71	3·24	4·97
8	B	5·66	13·85	2·48	0·04	9·96	0·69	3·14	3·20
9	O	1·29	2·66	2·35	0·04	3·82	1·73	−1·10	−2·89
10	C	5·88	13·93	2·51	0·03	7·06	0·60	3·34	6·29
11	C	5·88	13·95	2·62	0·04	8·92	0·84	3·22	4·19
12	O	1·29	2·66	2·32	0·05	3·01	1·56	−1·08	−1·91

Trial II—

Week No.	Supplement.	Intake gm. P.	Intake gm. Ca.	Outgo P Faeces.	Outgo P Urine.	Outgo Ca Faeces.	Outgo Ca Urine.	Balance gm. P.	Balance gm. Ca.
13	O	1·71	2·64	1·97	0·04	3·08	1·50	−0·30	1·94
14	A	6·26	13·88	2·12	0·04	4·93	0·57	4·10	8·38
15	A	6·26	13·93	2·44	0·04	4·74	0·44	3·78	8·75
16	O	1·71	2·65	2·02	0·03	2·29	1·71	−0·34	−1·35
17	B	5·93	13·47	1·91	0·03	7·06	0·50	3·99	5·81
18	B	5·93	13·44	2·05	0·04	6·56	0·30	3·84	6·58
19	O	1·71	2·68	1·97	0·03	2·61	0·90	−0·29	−0·83
20	C	6·24	13·44	1·87	0·04	2·56	0·37	4·33	6·51
21	C	6·24	13·41	1·91	0·05	6·08	0·30	4·28	7·03
22	O	1·71	2·64	1·35	0·03	1·98	1·54	0·33	−0·88

Trial III—

Week No.	Supplement.	Intake gm. P.	Intake gm. Ca.	Outgo P Faeces.	Outgo P Urine.	Outgo Ca Faeces.	Outgo Ca Urine.	Balance gm. P.	Balance gm. Ca.
23	O	1·71	2·65	1·73	0·03	2·07	1·54	−0·05	−0·96
24	A	6·26	13·39	1·90	0·04	7·38	0·47	4·32	5·54
25	A	6·26	13·38	1·89	0·03	7·14	0·45	4·34	5·79
26	O	1·65	2·65	2·65	0·04	4·54	0·89	−1·04	−2·78
27	B	5·86	13·44	2·57	0·04	7·66	0·71	3·25	5·07
28	B	5·96	13·45	2·50	0·04	6·66	0·50	3·32	6·29
29	O	1·65	2·63	2·32	0·03	3·18	0·74	−0·70	−1·29
30	C	6·13	13·44	2·25	0·04	8·78	0·34	2·84	4·32
31	C	6·13	13·42	2·03	0·03	6·22	0·42	4·07	6·80
32	O	1·65	2·66	2·19	0·03	4·36	0·49	−0·57	−2·19

Table VIII.

(a) Phosphorus.

Supplementary Phosphorus retained.

		A.			B.			C.			Mean.		
		B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.
Trial I.........	1st week.........	0·934	1·066	1·000	1·064	0·963	1·014	0·863	0·965	0·914	0·954	0·998	0·976
	2nd week........	*1·026*	*1·037*	*1·032*	*0·982*	*0·941*	*0·962*	*0·669*	*0·939*	*0·804*	*0·892*	*0·972*	*0·932*
	Mean............	0·980	1·052	1·016	1·023	0·952	0·988	0·766	0·952	0·859	0·923	0·985	0·954
Trial II........	1st week.........	0·960	0·971	0·966	1·050	1·019	1·035	1·070	1·024	1·047	1·023	1·005	1·016
	2nd week........	*0·916*	*0·901*	*0·909*	*0·988*	*0·983*	*0·986*	*0·998*	*1·013*	*1·006*	*0·997*	*0·966*	*0·967*
	Mean............	0·938	0·936	0·938	1·019	1·001	1·010	1·034	1·019	1·026	1·010	0·984	0·991
Trial III.......	1st week.........	1·106	1·070	1·088	0·818	0·979	0·899	1·004	0·777	0·891	0·976	0·942	0·959
	2nd week........	*1·079*	*1·075*	*1·077*	*0·800*	*0·995*	*0·898*	*0·929*	*1·051*	*0·990*	*0·936*	*1·040*	*0·988*
	Mean............	1·093	1·073	1·083	0·809	0·987	0·899	0·967	0·914	0·940	0·956	0·991	0·974
Mean...........	1st week.........	1·000	1·036	1·018	0·977	0·987	0·982	0·979	0·922	0·951	0·985	0·982	0·984
	2nd week........	*1·007*	*1·004*	*1·006*	*0·923*	*0·973*	*0·948*	*0·865*	*1·001*	*0·933*	*0·932*	*0·963*	*0·963*
	Mean............	1·004	1·020	1·012	0·950	0·980	0·965	0·922	0·962	0·942	0·959	0·988	0·974

TABLE IX.

Supplementary Calcium retained.

		A.			B.			C.			Mean.		
		B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.
Trial I.........	1st week.........	0·735	0·631	0·683	0·504	0·654	0·579	0·559	0·769	0·664	0·599	0·685	0·642
	2nd week........	*0·414*	*0·519*	*0·467*	*0·531*	*0·497*	*0·514*	*0·506*	*0·584*	*0·545*	*0·484*	*0·533*	*0·509*
	Mean............	0·575	0·575	0·575	0·518	0·576	0·547	0·533	0·676	0·605	0·542	0·609	0·575
Trial II........	1st week.........	0·880	0·890	0·885	0·821	0·638	0·730	0·771	0·682	0·730	0·824	0·737	0·782
	2nd week........	*0·831*	*0·834*	*0·833*	*0·615*	*0·709*	*0·662*	*0·556*	*0·731*	*0·644*	*0·667*	*0·758*	*0·713*
	Mean............	0·856	0·862	0·859	0·718	0·674	0·696	0·663	0·707	0·721	0·740	0·747	0·748
Trial III.......	1st week.........	0·978	0·686	0·832	0·681	0·657	0·669	0·778	0·561	0·670	0·812	0·635	0·724
	2nd week........	*0·762*	*0·709*	*0·736*	*0·781*	*0·770*	*0·776*	*0·694*	*0·791*	*0·743*	*0·746*	*0·757*	*0·751*
	Mean............	0·870	0·698	0·784	0·731	0·714	0·723	0·736	0·676	0·706	0·779	0·696	0·738
Mean...........	1st week.........	0·864	0·736	0·800	0·669	0·650	0·650	0·703	0·671	0·687	0·745	0·686	0·716
	2nd week........	*0·669*	*0·687*	*0·678*	*0·842*	*0·659*	*0·651*	*0·585*	*0·702*	*0·643*	*0·632*	*0·683*	*0·658*
	Mean............	0·767	0·712	0·739	0·656	0·654	0·655	0·644	0·686	0·664	0·689	0·684	0·687

Discussion.

The conduction of this experiment was identical with the previous one, except that the treatments and observations were repeated in three consecutive trials, making the data collected considerably more reliable and conclusive.

A statistical analysis of the results indicates that the retention of the phosphorus of di-sodium phosphate appears to be greater than that of V. Bonemeal while the retention of the phosphorus present in di-calcium phosphate is not significantly different from that of either di-sodium phosphate or that of V. Bonemeal.

TABLE VIII (*a*).

Mean Proportion of Phosphorus retained.

A.	B.	C.
Di-sodium phosphate.	*Di-calc. phosphate.*	*V. Bonemeal.*
1·012	0·965	0·942
	Percentage retention.	
100	96·5	94·2

TABLE IX (*a*).

Mean Proportion of Calcium retained.

A.	B.	C.
Di-sodium phosphate.	*Di-calc. phosphate.*	*V. Bonemeal.*
0·739	0·655	0·665
	Percentage retention.	
73·9	65·5	66·5

When the means for both animals and the three trials are taken the retention in the three supplements showed small variations as will be seen from the above table.

Remarkably high phosphorus retention figures were obtained for all three supplements.

As in the previous experiment the phosphorus of di-sodium phosphate was apparently completely retained.

Periods of maximum retention of phosphorus were not necessarily periods of maximum retention of calcium.

Both animals were consistently on a negative phosphorus and calcium balance during the periods when only the basal ration was given. Apparently therefore the available P and Ca of the basal ration was definitely below the maintenance requirements of the animals, while there is always the probability that what little P there was in the basal ration viz. 1·01 gm. was not necessarily in an easily available form; this view is strengthened by the high proportion of P voided in the faeces.

The consistent loss of Ca and P from the body during the periods when no supplement was fed i.e. when active resorption of skeleton took place, reveals that favourable conditions existed for maximum absorption of Ca and P during the periods when supplement was supplied, as the animal, starved for Ca and P, is obviously in a better position to retain these minerals than when the body is not in dire need of them.

The practical significance of this observation is important for it suggests that the greater the need of the animal for minerals the more economical use is made of the supply. In other words completely satisfying the mineral need of animals need not necessarily be, and probably is not, the most profitable way of feeding minerals under practical conditions. Furthermore, this observation throws light on the observation frequently made viz. that the stockman who feeds approximately half the phosphate required by his stock kept on P deficient pasture obtains well over that proportion of profit obtained by satisfying the total requirements. If a daily supplement of 3 oz. bonemeal per head produces a gain of 150 lb. in weight over 12 months then 1·5 oz. will produce well over 75 lb. (du Toit and Green, 1930).

The extraordinary high retention of P contained in the supplements given, was highly favoured by the low level of P intake and it would appear that whatever the digestive system converted into an absorbable form was absorbed and utilized; in the case of the water soluble sodium phosphate all was apparently absorbed and retained and even of the comparatively insoluble di-calcium phosphate and bonemeal very little was not digested and utilized by the animal. There was a tendency for the faecal P to increase during the periods of feeding bonemeal and di-calcium phosphate, again suggesting that the slight difference in retention between these two supplements and di-sodium phosphate was merely incomplete conversion of the former two substances into soluble and absorbable entities. It would be interesting to determine whether the difference in retention remains almost neglible at higher levels of supplementary intake.

Experiment I (c).

It was clear from Experiment I (*b*) that there was no difference in the efficiency of the calcium and phosphorus retention of the two animals when they were kept on the same level of mineral intake. Therefore in order to determine the retention of the phosphorus at different levels, the supplement for one animal was doubled.

A further trial, using the same animals as in Experiment I (*b*) was conducted in exactly the same way, as in the former three trials, to determine whether the levels of intake of the supplements affected the retention of P and Ca significantly. It is realized that the value of the results would be considerably reduced by placing only one animal on each of the two levels of intake but as other suitable animals were not available at the time and as these two animals showed no significant difference in utilizing P in the previous experiment, it was hoped that the results might produce indication, possible, of what could be expected.

A period of eight weeks elapsed from the end of the previous experiment and the commencement of this one. The animals were, however, kept under the same routine of feeding. At the end of the previous experiment the animals received for three weeks the same supplement of phosphorus in the form of V. Bonemeal as during the trials. For two weeks no supplement was fed, followed by three weeks of V. Bonemeal supplement. The animals were then fed the basal ration only for two weeks, during the latter of which faeces and urine were collected, this being the first week recorded in this experiment.

Bovine 5429 received the same amount of supplement as in the previous three trials and bovine 5436 was given twice that amount of supplement.

The mineral balances are recorded in Tables XXIV to XXVII.

TABLE X.

Mineral Balance (daily average).

Bovine 5429.

Supplement A = Na_2HPO_4 + $CaCO_3$ ie.. 4·54 gm. P + 11·27 gm. Ca.
B = $CaHPO_4$ + $CaCO_3$ i.e. 4·42 gm. P + 10·72 gm. Ca.
C = V. Bonemeal + $CaCO_3$ i.e. 4·47 gm. P + 11·40 gm. Ca.

Trial IV—

Week No.	Supplement.	Intake.		Outgo gm. P.		Outgo gm. Ca.		Balance.	
		gm. P.	gm. Ca.	Faeces.	Urine.	Faeces.	Urine.	gm. P.	gm. Ca.
33	O	2·05	2·80	2·35	·05	2·43	1·26	−0·35	−0·89
34	A	6·60	13·03	2·54	·05	4·78	0·31	4·01	7·94
35	A	6·59	13·17	2·64	·05	7·34	0·32	3·90	5·51
36	O	2·02	2·78	2·63	·04	1·50	2·09	−0·65	−0·81
37	B	6·45	13·46	2·77	·04	6·50	0·37	3·64	6·59
38	B	6·45	13·54	2·73	·04	5·60	0·32	3·68	7·62
39	O	2·04	2·72	2·58	·04	1·25	1·52	−0·58	−0·05
40	C	6·50	14·30	2·69	·04	8·12	0·53	3·77	5·65
41	C	6·52	14·16	3·24	·05	5·02	0·26	3·23	8·88
42	O	2·04	2·78	2·56	·05	1·50	1·12	−0·57	0·16

TABLE XI.

Mineral Balance (daily average).

Bovine 5436.

Supplement A = Na_2HPO_4 + $CaCO_3$ i.e. 9·08 gm. P + 22·54 gm. Ca.
B = $CaHPO_4$ + $CaCO_3$ i.e. 8·84 gm. P + 21·44 gm. Ca.
C = V. Bonemeal + $CaCO_3$ i.e. 8·94 gm. P + 22·80 gm. Ca.

Trial IV—

Week No.	Supplement.	Intake.		Outgo gm. P.		Outgo gm. Ca.		Balance.	
		gm. P.	gm. Ca.	Faeces.	Urine.	Faeces.	Urine.	gm. P.	gm. Ca.
33	O	2·05	2·74	2·55	·05	2·41	1·08	−0·55	−0·75
34	A	11·14	25·32	3·80	·05	13·70	·40	7·29	11·22
35	A	11·13	25·34	4·40	·05	14·72	·37	6·64	10·25
36	O	2·02	2·72	2·77	·04	1·97	·79	−0·79	−0·04
37	B	10·85	24·34	4·60	·04	13·92	·37	6·21	10·05
38	B	10·85	24·32	3·90	·05	14·19	·34	6·90	9·79
39	O	2·04	2·76	3·17	·04	1·92	·59	−1·17	−0·25
40	C	10·97	25·58	4·24	·04	14·62	·36	6·69	10·60
41	C	10·97	25·62	4·68	·04	16·15	·34	6·25	9·13
42	O	2·04	2·90	2·72	·05	2·26	·94	−0·73	−0·30

TABLE XII.

Supplementary Phosphorus retained.

		A.			B.			C.			Mean.		
		B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.
Trial IV........	1st week.........	0·993	0·877	0·935	0·968	0·813	0·891	0·973	0·855	0·914	0·978	0·848	0·913
	2nd week........	*0·969*	*0·805*	*0·887*	*0·977*	*0·891*	*0·934*	*0·852*	*0·805*	*0·829*	*0·933*	*0·834*	*0·883*
	Mean............	0·981	0·841	0·911	0·973	0·852	0·913	0·913	0·830	0·872	0·956	0·841	0·898

TABLE XIII.

Supplementary Calcium retained.

		A.			B.			C.			Mean.		
		B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.
Trial IV........	1st week.........	0·780	0·516	0·648	0·655	0·476	0·566	0·486	0·477	0·482	0·640	0·490	0·565
	2nd week........	*0·764*	*0·472*	*0·618*	*0·751*	*0·464*	*0·608*	*0·769*	*0·413*	*0·591*	*0·761*	*0·450*	*0·606*
	Mean............	0·772	0·494	0·633	0·703	0·470	0·587	0·628	0·445	0·536	0·701	0·470	0·585

Discussion.

The mean proportion of P and Ca retained by the two animals are presented in Table XXVIII and a summary of the data in Table XIV. Animal No. 5429 received 4·5 gm. P and 11·15 gm. Ca and No. 5436 8·9 gm. P and 22·3 gm. Ca in their supplement. Di-sodium phosphate, di-calcium phosphate and V. bonemeal were fed durings periods A, B, and C respectively.

TABLE XIV.

Bovine No.	Supplement.		A.		B.		C.	
	P.	Ca.	P.	Ca.	P.	Ca.	P.	Ca.
5429........	4·5	11·1	98·1	71·2	97·3	70·3	91·3	62·8
5436........	8·9	22 3	84·1	94 4	85·2	47 0	83·0	44·5

A glance at Table XIV reveals a remarkably high retention of Ca and P by both animals. Furthermore there was no significant difference between the retention of P or Ca for either animal when the supplement was given as di-sodium phosphate or calcium phosphate or V. bonemeal. It would appear, therefore, that these animals were able at both the levels of intake to "digest", absorb and retain these two minerals equally well whether they were supplied as the water soluble di-sodium phosphate or the relatively insoluble di-calcium phosphate or V. bonemeal. This result is remarkable and tends to strengthen the inference made in the discussion of the last experiment viz. that the phosphorus contained in di-sodium phosphate, di-calcium phosphate and in V. bonemeal could be regarded for practical purposes as being made equally available to the animal body during digestion.

The retention of both Ca and P was reduced at the high levels of intake; P decreased from approximately 95 per cent. retention to 84 per cent. and Ca from about 70 to 45 per cent. There is the possibility of course that the higher level of mineral intake exceeded the mineral requirement of the animals and that the reduction in retention was due to the elimination of the P and Ca present in excess of the requirement. In that case appreciable differences in the availability of P or Ca in these supplements fed might be completely masked by the excess present, unless, of course, the product was so poorly available that the P or Ca provided during absorption did not satisfy the requirement of the animal. The supplements fed showed only slight differences at the lower level of intake and indicated therefore that the P and Ca they contained were approximately equally available to the animals at that level. Furthermore, the higher level at which both P and Ca were fed is certainly not less than that accepted for the P and Ca requirement

for the type of animal used, and one is forced to the conclusion, that the reduction in percentage retention at the higher level of intake is mainly due to the elimination of the Ca and P present in excess of the quantities required by the animals, and not necessarily to the inavailability of these minerals in the products fed. As a matter of fact a true picture of the availability of Ca and P present in different products cannot be obtained when the latter is given at levels that are in excess of the requirements of the experimental animals.

Summarizing the results obtained in the experiments on the availability of P in di-sodium phosphate, di-calcium phosphate and bonemeal one is struck by the remarkably high retention of P at the lower level of intake of P viz. 4·5 gm. Over 90 per cent. P was retained in the animal body, while even when the intake of P was doubled, which probably slightly exceeded the P requirements of the animals more than 80 per cent. of the P ingested was retained. Sodium phosphate appeared to possess a slight advantage over the other phosphates in that in two experiments slightly more of its P was retained than of that given as di-calcium phosphate and as bonemeal; in the third experiment where only one animal was used no difference existed between the quantities of P retained when di-sodium phosphate or di-calcium phosphate was fed.

V. EXPERIMENT II.

THE UTILIZATION OF CALCIUM AND PHOSPHORUS BY BOVINES ON RATIONS CONTAINING DIFFERENT LEVELS OF MINERAL INTAKE WITH A CONSTANT CALCIUM TO PHOSPHORUS RATIO.

Animals:—Ten half-grade Friesland steers of the following ages and weights were used in this experiment.

At commencement of experiment: 24.6.33.

Group.	Bovine No.	Age months.	Weight.
			lb.
I	4712	17	821
	3478	18	800
II	3480	18	812
	3465	18	767
III	3464	18	798
	3467	18	818
IV	3454	18	756
	3456	18	836
	Introduced into Expt. 9/7/34.		
V	5430	12	450
	5431	12	400

The animals were divided into five groups of two animals each. Group V was introduced into the experiment 12½ months after the commencement.

Ration of Groups I-IV.

Feed.	Amount.	Percentage P.	P gm.	Percentage Ca.	Ca gm.
Samp	3·5 Kg.	·039	1·37	·071	·25
Hay	1·2 Kg.	·105	1·26	·297	3·56
Meatmeal	·24 Kg.	·808	1·94	·820	1·97
Water	± 10 litres	—	—	—	·50
Sodium chloride	15 gm.	—	—	—	—
Total mineral intake	—	4·57 gm. P	and 6·38	gm. Ca.	

Three months after the commencement of the experiment ·5 Kg. green feed was added to the ration. Towards the end of the experiment, i.e. from the 22nd month onwards the basal ration was increased to 4·5 Kg. samp, 2 Kg. hay and meatmeal was substituted for blood meal. The total mineral intake was not affected by these changes.

The four groups of animals were given the basal ration for six weeks, the mineral balance being determined on the ration during the last two weeks. The supplements were then given continuously until the end of the experiment.

The following Table shows the amount of calcium and phosphorus which was added as di-calcium phosphate and calcium carbonate to the basal ration of each group.

TABLE XV.

Calcium and phosphorus added to basal ration.

Group.	Basal Ration.		Added.		Total Intake.		Total Intake.
	Ca gm.	P gm.	Ca gm.	P gm.	Ca gm.	P gm.	Ca : P ratio.
I	6·32	4·51	9·14	3·49	15·46	8·00	1·93 : 1
II	6 32	4·51	5·21	1·28	11·53	5·85	1·97 : 1
III	6·32	4·56	18·10	1·28	24·42	5·85	4·18 : 1
IV	6·32	4·54	2·38	—	8·70	4·54	1·92 : 1
V	2·14	1·01	—	—	2·14	1·01	2·12 : 1

From the above Table it will be seen that the Ca : P ratio of groups I, II, IV and V was about 2·0 : 1, whereas for group III, the ratio was 4 :1, the level of phosphorus intake being the same as that of group II.

At a later date group V was included in the experiment in order to determine to what extent bovines could utilize the calcium and phosphorus of a ration containing a lower mineral content than that supplied to group IV.

Ration of Group V.

Feed.	Amount.	Percentage P.	P gm.	Percentage Ca.	Ca gm.
Samp........................	1·50 Kg.	·037	·56	·015	·23
Hay..........................	·25 Kg.	·039	·10	·240	·60
Blood meal.................	·10 Kg.	·109	·11	·136	·14
Green feed..................	·50 Kg.	·047	·24	·128	·64
Water.......................	± 10 litres	—	—	—	—
Salt mixture A..............	15 gm.	—	—	—	—
Total mineral intake.........	—	—	1·01 gm.	P and 2·14	gm. Ca.

The ration of group V was the same as that given to bovines 5429 and 5436 in Experiment I (*b*). As in the latter case, the samp, hay and blood meal was finally doubled. It thus became:—

3·0 Kg. Samp.

0·5 Kg. Hay.

0·2 Kg. Blood meal.

0·5 Kg. Green feed.

15 gm. Salt mixture A.

The Ca : P ratio under the above conditions showed a slight alteration, but it was decided not to add minerals to the ration in order to bring the ratio nearer to 2·0 : 1.

In both animals of each group the mineral balances were determined (commencing with group V at the 15th month of the experiment) for one week monthly, the calcium and inorganic phosphorus content of the blood being determined at monthly intervals. In the case of group V the plasma phosphatase was also determined at the above intervals. The body weights and detailed results of the balance trials are given in the appendix in Tables XXIV to XXXIV which have been summarized in Table XIX. The P and Ca of the blood and bone analyses are given in Tables XVI, XVII and XX respectively.

TABLE XVI.

Inorganic Blood Phosphorus in mg. per 100 c.c. blood.

Month of Expt.	Bovines.									
	4712.	3478.	3480.	3465.	3464.	3467.	3454.	3456.	5430.	5431.
4	7·6	6·9	7·0	7·2	7·2	6·9	6·6	6·0	—	—
5	7·3	7·1	7·2	7·0	6·5	6·4	6·5	6·2	—	—
6	6·4	7·4	6·4	6·9	7·0	6·2	6·0	5·2	—	—
7	7·2	8·0	6·6	5·8	6·4	5·8	5·2	4·3	—	—
8	6·8	6·8	5·8	5·4	5·9	5·6	4·6	3·9	—	—
9	5·8	7·2	5·2	5·0	4·8	5·0	4·3	3·3	—	—
10	6·2	6·6	5·6	5·2	5·0	4·2	—	3·6	—	—
11	5·8	7·2	4·6	4·9	3·6	3·5	—	3·2	—	—
12	5·4	7·6	4·8	—	3·9	3·7	—	3·0	—	—
13	5·2	7·7	4·4	—	3·4	3·4	—	2·7	—	—
14	5·3	—	3·0	—	2·8	2·6	—	2·5	—	—
15	6·1	—	3·5	—	3·2	2·9	—	2·9	7·0	4·4
16	6·2	—	3·7	—	3·3	3·4	—	2·6	6·4	5·0
17	6·2	—	3·9	—	3·4	3·1	—	2·7	5·5	4·0
18	6·2	—	4·7	—	3·5	2·7	—	2·8	4·4	3·2
19	6·1	—	4·6	—	4·0	—	—	2·9	4·8	3·6
20	5·7	—	4·8	—	3·6	—	—	2·8	4·7	3·5
21	6·0	—	5·1	—	3·4	—	—	2·8	3·5	2·3
22	5·7	—	4·7	—	3·6	—	—	2·9	3·4	3·0
23	5·6	—	4·3	—	3·4	—	—	2·7	3·2	2·6
24	5·3	—	4·0	—	3·5	—	—	2·9	3·3	2·6
25	5·2	—	3·9	—	3·4	—	—	2·8	3·1	1·8
26	5·0	—	3·5	—	3·3	—	—	2·9	2·9	1·6

TABLE XVII.

Blood Calcium in mg. per 100 c.c. blood.

Month of Expt.	Bovines.									
	4712.	3478.	3480.	3465.	3464.	3467.	3454.	3456.	5430.	5431.
4	10·2	10·6	10·0	9·9	10·2	9·8	10·4	1·0	—	—
5	10·0	10·2	9·8	10·0	9·8	10·2	9·6	8·9	—	—
6	9·6	10·0	8·9	10·1	10·4	10·0	8·9	9·2	—	—
7	10·4	10·6	10·6	11·2	11·2	9·3	10·0	10·8	—	—
8	10·2	9·9	10·2	10·6	10·8	9·4	9·4	10·4	—	—
9	9·8	10·4	10·0	10·4	10·4	9·5	10·2	10·0	—	—
10	10·0	10·0	10·1	10·7	10·3	9·8	—	9·6	—	—
11	9·4	10·6	9·8	10·5	10·7	9·7	—	9·2	—	—
12	10·6	9·8	10·0	—	10·1	10·0	—	9·0	—	—
13	10·4	10·0	9·6	—	10·0	9·6	—	8·0	—	—
14	9·4	—	9·5	—	10·6	9·5	—	10·4	—	—
15	9·8	—	10·0	—	10·7	10·3	—	11·2	10·8	10·5
16	8·9	—	10·2	—	10·4	11·2	—	10·6	10·4	10·7
17	10·6	—	10·1	—	8·9	10·8	—	10·4	10·6	10·2
18	10·2	—	10·4	—	9·6	11·0	—	8·4	10·4	10·5
19	10·0	—	10·6	—	10·4	—	—	9·4	10·2	10·0
20	10·0	—	10·3	—	8·6	—	—	10·6	10·2	10·1
21	10·2	—	9·7	—	10·4	—	—	8·6	10·9	10·6
22	9·8	—	10·6	—	10·2	—	—	10·4	8·9	9·6
23	9·6	—	9·5	—	10·9	—	—	10·2	10·0	11·0
24	10·1	—	10·0	—	10·0	—	—	10·0	8·0	8·9
25	9·8	—	10·2	—	9·9	—	—	8·4	8·6	8·9
26	8·9	—	9·1	—	9·6	—	—	9·0	9·1	8·4

Table XVIII.

Plasma Phosphatase in Bodansky Units.

Bovine.	Month of Experiment.				
	22.	23.	24.	25.	26.
5430......................	5·2	4·8	6·9	6·8	8·0
5431......................	6 8	8·2	8·0	10·9	11·3

Table XIX.

Mean Daily Mineral Intake and Retention in gm.

(Summary of Tables XXIV to XXXII.)

Group.	Animal.	Phosphorus.		Time on experimental ration.	Calcium.	
		Intake.	Retention.		Intake.	Retention.
I...	4712	8·34	4·42	3rd–25th month expt.	16·36	6·36
	3478	8·59	4·57	3rd–13th month expt.	16·35	7·19
II...	3480	5·83	2·47	3rd–25th month expt.	12·09	3·67
	3465	6·42	2·14	3rd–12th month expt.	12·62	4·13
III...	3464	5·48	2·03	3rd–25th month expt.	24·09	5·09
	3467	5·79	2·18	3rd–18th month expt.	24·42	4·23
IV...	3454	4·80	1·20	3rd–10th month expt.	8·96	2·49
	3456	3·64	1·07	3rd–25th month expt.	7·94	1·78
V...	5430	1·68	−0·11	15th–26th month expt.	2·70	−0·54
	5431	1·68	−0·05	15th–26th month expt.	2·70	−0·35

Discussion.

From weeks Nos. 1 and 2, in the mineral balance tables it will be seen, that the animals of groups I to IV while receiving the basal ration (without supplements) reacted uniformly with regard to the retention of calcium and phosphorus. They were on a negative calcium balance, while the phosphorus balance varied from slightly negative to slightly positive.

TABLE XX.

Bone Analysis.

Bone.	Group.	Bovine.	Green Weight.	Green Bone.				Breaking Strength per sq. in. 6 in. span.	Dry fat free bone.			Percentage Ash of green weight.
				Percentage Water.	Percentage Fat.	Percentage Dry fat-free.	Sp. Gravity.		Percentage Ash.	Percentage Ca.	Percentage P.	
Femur...........	I	4712	2,470	13·1	35·2	51·7	1·240	2,900	65·5	38·7	18·2	33·8
	II	3480	2,555	17·0	38·4	44·6	1·245	1,800	61·7	38·9	17·7	27·5
	III	3464	2,374	16·4	40·8	42·8	1·174	1,550	61·5	39·5	17·8	26·3
	VI	3456	2,385	21·4	41·3	37·3	1·150	3.120	57·0	39·7	17·2	21·3
	V	5431	1,205	14·7	44·6	40·7	1·205	1,600	58·9	38·6	17·1	24·1
Humerus.........	I	4712	2,075	13·1	38·6	48·3	1·200	1,500	64·9	39·1	18·1	31·3
	II	3480	1,876	17·3	44·2	38·5	1·225	1,350	61·9	39·7	17·4	23·3
	III	3464	1,916	17·5	42·5	40·0	1·230	2,200	61·6	39·6	17·5	24·6
	IV	3456	1,840	23·0	40·5	36·5	1·150	2,010	55·0	39·4	17·0	20·1
	V	5431	867	17·0	43·8	39·2	1·120	1,400	58·7	38·0	17·2	23·0
Metacarpus......	I	4712	598	11·3	22·9	65·8	1·410	3,740	67·1	39·7	18·0	44·1
	II	3480	505	17·6	25·7	56·7	1·345	2,695	65·2	38·6	17·7	37·0
	III	3464	562	18·2	24·3	57·5	1·400	2,850	65·9	38·7	17·7	37·9
	IV	3456	520	20·7	26·5	52·8	1·300	1,680	59·1	39·8	17·9	31·4
	V	5431	282	17·0	28·4	54·6	1·310	1,250	61·9	39·1	17·8	33·8
Sixth Right Rib..	I	4712	347	27·10	19·0	53·90	1·422	—	62·0	—	—	33·3
	II	3480	307	30·35	19·92	49·73	1·296	—	57·3	—	—	28·6
	III	3464	268	27·49	19·28	53·23	1·340	—	58·6	—	—	31·3
	IV	3456	251	35·46	23·11	41·43	1·141	—	56·8	—	—	3·5
	V	5431	138	27·81	26·54	45·65	1·212	—	52·3	—	—	23·9

Group I, Bovines 4712 *and* 3478.

The average daily mineral intake of this group was 8·47 gm. P and 16·36 gm. Ca.

The monthly weights of the animals of group I (Table XXIV), indicate that the basal ration permitted neither maximum growth nor the putting on of extra fat. The animals ate greedily, readily finishing their ration and licking their troughs. If they had been given food *ad lib.* they would certainly have eaten much more and gained considerably in weight. The food intake was limited by the group showing the lowest consumption.

During the first twelve months of the experiment the average gain in weight was just over 200 lb. At this stage bovine 3478 died of acute haemorrhagic gastro-enteritis. Bovine 4712 gained 250 lb. from the 14th to the 26th month of the experiment.

The Ca : P ratio of the ration may be considered as optimal for growing cattle. The phosphorus contained in the ration was well utilized, the average daily retention being 4·5 gm. P or 53 per cent. of the total intake, while 6·7 gm. Ca, that is, 41·5 per cent. of the calcium was retained.

The inorganic phosphorus and the calcium content of the blood remained within normal limits while the blood calcium values are of no significance in this experiment. The inorganic phosphorus values suggest sufficiency of P in the ration. It must, however, be pointed out that over 40 per cent. of the total phosphorus of the ration was given in inorganic form which was highly available.

From Table XX it is seen that for each of the four bones examined, the green weight and the percentage of dry-fat-free bone were greater, while the percentages of water and fat were less in the bones of group I than in those of any other group. As the specific gravity values of the bone fluctuate no definite conclusions can be drawn from them.

With the type of machine used, it was exceedingly difficult to keep the femurs and humeri in the required position while determining their breaking strength and the values obtained cannot be regarded as presenting a true indication of the breaking strength of these bones. The metacarpus whose shape prevents undue slipping was found to be most suitable for this work.

The metacarpus of the animal of group I registered 3,740 lb. per square inch over a six inch span, this figure being considerably higher than that for the same bone in any of the other groups.

The proportion of ash in the dry-fat-free bone shows its highest values for the bones of group I, the percentage of ash of the metacarpus, rib, humerus and femur being 67·1, 62·0, 64·9 and 65·5 respectively.

The calcium and phosphorus content of the ash, of the different bones of the animals representing the different groups remained practically constant, the Ca : P ratio of the ash oscillating very closely round 2·2 : 1.

The most striking variation was obtained when the percentage was calculated on the green weight of the bones. This value was considerably higher for the bones of group I. Of the metacarpus almost half and of the femur, humerus and rib one-third of the green bone is ash.

Group II, Bovines 3480 *and* 3465.

The average daily mineral intake was 6·13 gm. P and 12·35 gm. Ca.

The animals of this group reacted disappointingly. At times they lost appetite, leaving some of the basal ration. During the 13th month of the experiment Bovine 3465 became suddenly sick and died of renal calculi. During the 6th month of the experiment Bovine 3480 became sick and did not recover completely before the 11th month. At that time there was no steer of the same age available on the station to replace it, and there was no alternative but to continue the experiment with only this animal in group V.

It decreased to its initial weight during the time it was sick. After recovery from the 11th month onwards it consumed its full ration satisfactorily and for the last twelve months of the experiment it gained as much in weight as the animal in group I for the same period.

The average daily retention of calcium and phosphorus for the total experimental period was 3·9 gm. and 2·3 gm. respectively, being 31·8 per cent. of the calcium and 37·5 per cent. of the phosphorus intake.

The inorganic blood phosphorus values leave no doubt that the phosphorus intake and, consequently, the retention under these conditions were below the optimal requirement of the animals. The blood calcium values remained normal, being in the vicinity of 11 mgm. per 100 c.c. blood.

The weights of the green bones of the animals in this group compared favourably with those in group I. The percentages of water and of fat in the bones were definitely higher, while the percentages of dry-fat-free bone and of ash were lower than those of group I. The breaking strength of the metacarpus registered more than a thousand pounds less, and the percentages of ash were about 6 per cent. less, than in the previous group.

Group III, Bovines 3464 *and* 3467.

The average daily mineral intake of this group was 24·26 gm. Ca and 5·64 gm. P, with a Ca : P ratio of 4·18 : 1 which was double that of any other group.

During the fourth month after commencement of the experiment these animals suffered from loss of appetite to such an extent that Bovine 3464 after eight months weighed almost the same as at the beginning. During the second half of the experimental period the appetite improved and the animal gained in weight. Bovine 3467 behaved slightly better, but it unfortunately died of anaplasmosis after 18 months in the experiment.

The average daily retention was 2·1 gm. P and 4·6 gm. Ca, being 37·2 per cent. of the phosphorus and 19·2 per cent. of the calcium of the ration. Both these animals showed about the same retention of phosphorus as the previous group, but a slightly higher retention of calcium.

The inorganic blood phosphorus values of about 3·5 mgm. per 100 c.c. were below normal, indicating that a daily retention of 2·1 gm. P was insufficient for growing bovines.

The fact that the inorganic blood phosphorus values of this group were slightly lower than those of group III may indicate that the increased addition of calcium carbonate to the rations of group III had a detrimental effect upon phosphorus metabolism.

There is hardly any difference in the chemical analysis of the bones of the animals of group II and group III in spite of the more favourable ratio of Ca : P in the former group. The breaking strength of the metacarpus of group III was slightly higher than that of group II.

Group IV, Bovines 3454 and 3456.

The daily ration of this group contained 8·5 gm. Ca and 4·2 gm. P.

Bovine 3454 ate very well without showing any signs of loss of appetite. It gained 127 lb. during the first eight months, this being equal to the weight increase of the animals in group I.

The average daily retention of Bovine 3454 was 2·49 gm. Ca and 1·20 gm. P, this being 27·8 per cent. and 25·0 per cent. of the total intake respectively. The Ca : P ratio of the minerals retained was 2·08 : 1. Unfortunately this animal had to be destroyed in the 10th month of the experiment, on account of a fracture of the femur. Its inorganic blood phosphorus at this time showed signs of a phosphorus deficiency in the ration.

Bovine 3456, the surviving animal, went off feed almost at the beginning of the experiment and could not be induced to consume the full ration. Therefore the samp of the ration was reduced to 2·5 Kg. and the hay to 0·7 Kg., giving an average daily intake of 3·64 gm. P and 7·94 gm. Ca. The average daily retention was 1·14 gm. P and 2·14 gm. Ca, or 27 per cent. and 25 per cent. of the total intake respectively. The Ca : P ratio of the minerals retained was 1·88 : 1. Owing to the reduced ration, this animal gained only 50 lb. in weight for the entire experimental period.

The inorganic phosphorus of the blood soon dropped below 3 mgm. per 100 c.c. and remained at this level. The calcium content of the blood was not influenced by the low level of mineral intake but remained normal.

The green weights of the bones were on the average slightly less than those of the other groups. Calcification, however, was very incomplete, more so than in the three previous groups. For each of the four bones examined in this group, the percentage of water and fat was high, while that of the dry-fat-free bone was low, the specific gravity also being low. The metacarpal break registered 1,680 lb.

per square inch, being about 1,000 lb. lower than that of group II, and about 2,000 lb. lower than that of group I. The percentage of ash in the green femur, humerus and rib was just over 20, as against 30 per cent, for that of group I. The percentage ash of the green bones of the last-mentioned group ,was 1½ times that of group IV.

Group V. Bovines 5430 *and* 5431.

Average daily mineral intake: 1·68 gm. P and 2·7 gm. Ca.

These two animals were given a basal ration as low as possible in Ca and P. The total daily P and Ca present was only about 1·6 gm. of each. Hence it is not surprising that those quantities were insufficient for maintenance as reference to Table XXXII will indicate.

These animals were in negative calcium and phosphorus balances, and only after about seven months did their balances gradually become slightly positive. The average mineral balance for the entire period, however, was negative for both animals. The inorganic blood phosphorus showed that these animals were suffering from severe aphosphorosis, the blood phosphatase values in Table XVIII further indicate a disturbed calcification of the bones.

Although the animals consumed the whole ration and appeared to be in good health, they gained only about 200 lb. each, during the 13 months in which they were in the experiment. It is, however, doubtful whether they would have consumed more of this mineral ration, if the quantity had been increased.

At the end of the experiment one animal, viz, bovine 5431, was destroyed, and bones collected for analysis. The green bones weighed considerably less than those of the previous groups, which could not be regarded as suitable controls on account of the remarkable differences in age between group 5 and the others.

Except for the femur the specific gravity of the bones was very low.

The breaking strength of the metacarpus registered only 1,250 lb. per square inch. The percentage of dry fat-free bone, its percentage ash and the percentage ash of the green bone were low.

General.

Before the animals were placed in the experiment, and fed the basal ration, which was deficient in both calcium and phosphorus, they had adequate minerals to supply the tissues with their normal requirements. With the change to the mineral deficient rations, a state of negative calcium and phosphorus balance ensued, the mineral intake sinking below the level of excretion, resulting in a direct loss to the tissues.

The addition of supplements to the basal ration resulted in a moderate retention of the minerals in group I. As the levels of mineral intake were decreased below the optimal requirements (groups II to V) the total retention decreased, those animals at the lowest level of mineral intake (group V) being in negative balances.

Less efficient retention of the minerals was observed in Experiment I (C) at the higher than at the optimal level of mineral intake. In this experiment the efficiency of retention of the mineral decreased as the mineral intake was diminished below the optimum. In both experiments the maximum percentage retention was found at the optimal level of mineral intake.

During the periods when only the basal ration was fed and in the group at the lowest level of phosphorus intake, over 50 per cent. of the total calcium consumed might be excreted in the urine. It was noted in group V (deficient Ca and P) that as the calcium balance eventually became positive the calcium content of the urine decreased. The urine always contained minimal amounts of phosphorus, irrespective of the level of intake.

As the level of mineral intake was lowered from group I to group V the inorganic phosphorus of the blood decreased. The values of the animals of group I alone may be considered as normal, whereas those of bovine 5431 (group V), finally decreasing to the low level of 1·6 mgm. per 100 c.c. blood indicate that this animal was suffering from severe aphosphorosis.

The blood calcium values remained normal irrespective of the level of mineral intake.

The animals of group I, fed adequate minerals, consumed their full rations, eating greedily, but as the level of mineral intake was lowered loss of appetite became significant in groups II to IV.

In group V, fed a reduced ration and in experiment for only 13 months, symptoms of anoxeria were less pronounced.

The results obtained from the bone studies show that the level of mineral intake had a marked effect upon the breaking strength of the metacarpus, the percentage of dry-fat-free bone, its ash content and the percentage of ash in the green bone. It is apparent that the calcium and phosphorus content of the ration may serve as limiting factors in bone calcification, gravities, and the breaking strengths of the femurs and humeri.

Bovine 5431 of group V being younger and weighing less, the green weights and measurements of its bones were, as expected, smaller than those of the other groups.

The findings of the histological examinations of the bones are discussed in a paper by Theiler *et al* (1937).

From a critical study of the results obtained in this experiment it would appear that the animals in group I, receiving daily 8·5 gm. P and 16·4 gm. Ca received an adequate supply of these two minerals. A reduction in P intake to approximately 6 gm. whether the Ca be reduced proportionately or not, caused relatively poorer utilization of P; whereas 4·5 gm. P was retained when 8·4 gm. was given, approximately 2·3 gm. was retained when about 5·8 gm. was supplied. The ratio of Ca to P was apparently without significant effect on the retention of P. Group IV received no supplementary P. Approximately 4 gm. was present in the basal ration, of which about

half was retained. A reduction of P in group V to 1·68 gm. brought about a continual loss of P from the body. It is remarkable that growth could have taken place at all under these conditions.

The percentage ash of the bones affords an excellent index of the degree of calcification. This figure varied from 44·1 per cent. for the metacarpus of the animal receiving adequate Ca and P to 31·4 per cent. for that in the mineral deficient group. The breaking strength of the metacarpus affords confirmatory evidence for the conclusions based on ash determinations.

Increasing demands for minerals by the animals fed mineral deficient rations may diminish losses by more economical re-utilization of endogenous minerals and hence reduce excretion in the later as compared with the earlier stages of the experiment and so abolish a negative mineral balance.

VI. EXPERIMENT III.

Animals.—For this experiment twelve young Friesland heifers were specially selected for uniformity of age and conformation. They were divided into six groups of two each, as follows:—

Commencement of Experiment: October, 1933.

Group.	Bovine.	Age, months.	Weight.
			lb.
I	5154	16	640
	5163	15	600
II	5155	16	580
	5157	16	570
III	5158	16	570
	5159	16	620
IV	5160	16	650
	5161	15½	600
V	5153	16	670
	5167	11½	510
VI	5147	16½	580
	5149	16½	570

Basal Ration.

At the commencement of the experiment these animals were given a basal ration consisting of:—

3·0 Kg. Fanko (maize endosperm).
·5 Kg. Hay.
·5 Kg. Green feed.
·25 Kg. Meat meal.
25 gm. Salt mixture A.
Rain water *ad lib.*

This ration supplied 4 gm. P and 4·5 gm. Ca. Six weeks after the commencement of the experiment two consecutive mineral balances were determined, being weeks Nos. 1 and 2 in the mineral balance tables.

After these mineral balances had been determined the rations of the animals were supplemented with di-sodium phosphate and calcium carbonate according to the following table:—

TABLE XXI.

Group.	Bovine No.	Basal Ration.		Supplement.		Total Intake.		Ca : P ratio of total intake.
		Ca gm.	P gm.	Ca gm.	P gm.	Ca gm.	P gm.	
I......	5154	4·52	4·02	18·4	7·0	22·92	11·02	2·08 : 1
	5163	4·52	4·02	18·4	7·0	22·92	11·02	2·08 : 1
II......	5155	4·52	4·02	18·4	—	22·92	4·02	5·72 : 1
	5157	4·52	4·02	18·4	—	22·92	4·02	5·72 : 1
III......	5158	4·52	4·02	40·0	—	44·52	4·02	11·1 : 1
	5159	4·52	4·02	40·0	—	44·52	4·02	11·1 : 1
IV......	5160	4·52	4·02	—	—	4·52	4·02	1·12 : 1
	5161	4·52	4·02	—	—	4·52	4·02	1·12 : 1
V......	5153	4·52	4·02	40·0	7·0	44·52	11·02	4·04 : 1
	5167	4·52	4·02	40·0	7·0	44·52	11·02	4·04 : 1
VI......	5147	4·52	4·02	—	7·0	4·52	11·02	0·41 : 1
	5149	4·52	4·02	—	7·0	4·52	11·02	0·41 : 1

From the beginning of the 8th month the basal ration was increased and modified as follows:—

TABLE XXII.

Basal Ration.

Feed.	Amount.	Percentage P.	P gm.	Percentage Ca.	Ca gm.
Samp......................	4·0 Kg.	·059	2·36	·007	·29
Hay......................	·5 Kg.	·105	·525	·286	1·43
Green feed..................	·5 Kg.	·0385	·192	·070	·35
Meat Meal..................	·15 Kg.	·81	1·21	·971	1·46
Tap water..................	± 10 litres	—	—	·005	·50
Salt Mixture A............	25 gm.	—	—	—	—
Total Intake................	—	—	4·29 gm. P.	—	4·03 gm Ca.

The animals were given this basal ration for the rest of the experimental period. Variations in water consumption and in the analyses of the feeds caused slight variations in the mineral intake of the animals.

From the 9th month onwards, i.e. seven months after minerals had been added to the rations, mineral balance trials were carried out regularly for a period of one week every month, until the animals were killed for the purpose of collecting the bones for chemical and histological studies. The mineral balance of only one animal in each group is recorded, although frequently the mineral balance of both animals in any particular group was determined. These analyses are not reported as they were mainly used as a check.

The animals of groups II and III could not be induced to consume the full ration, those of group III being worse in this respect During the first 14 months of the experiment it was often necessary to reduce the ration of group II to 2 Kg. samp instead of 4 Kg. for a week or two. As soon as the animals consumed the 2 Kg. samp ration satisfactorily, they were again given 4 Kg. From the 15th month onwards, however, it became necessary to feed 3 Kg. samp daily.

Eight months after the commencement of the experiment the samp in the ration of group III was reduced to 2 Kg. A month later the total basal ration was given for three weeks, when the samp had again to be reduced to 2 Kg. at which level it remained until the end of the experiment.

Bovine 5159 of group III died in the 13th month and bovine 5161 of group IV in the 19th month of the experiment.

Bovine 5158, the surviving animal of group III, was in a very poor condition and consumed the decreased basal ration with difficulty. In the 18th month of the experiment 1·07 gm. P as di-sodium phosphate, was added to the basal ration, partly to save the animal and partly in order to supplement the phosphorus lost in the 2 Kg. samp by which the ration was reduced.

In group II ·53 gm. P in the form of di-sodium phosphate was added to the ration of bovines 5155 and 5157, this representing the amount present in 1 Kg. of samp, by which the basal ration was reduced.

The animals received these additions of phosphorus just after they had been bled in the 18th month, that being approximately fourteen days before the mineral balance trial had to be conducted. The supplementary phosphate feeding was discontinued after the bleeding at the beginning of the 21st month.

Detailed results of the balance trials are given in the appendix in Tables XL-LI, a summary of which appears in Table XXIII.

Tables XXXV-XXXIX representing the body weights and values for blood calcium appear in the appendix.

TABLE XXIII.

Daily Mean Mineral Intake and Retention.

Summary of Tables XL to LI.

Group.	Bovine.	Intake.		Mean Ca : P ratio of ration.	Balance.		Percentage retention.	
		Ca gm.	P gm.		Ca gm.	P gm.	Ca.	P.
I......	5154	22·91	10·49	2·18 : 1	6·69	6·58	42·3	62·6
II......	5157	22·61	3·44	6·57 : 1	4·38	·35	19·4	10·2
III......	5158	43·30	3·03	14·26 : 1	10·58	·68	24·4	22·4
IV......	5160	4·23	3·44	1·23 : 1	0·14	·76	—	22·0
V......	5153	45·00	10·68	4·21 : 1	14·76	5·31	32·8	49·8
VI......	5149	4·14	10·68	·39 : 1	1·12	4·29	27·0	40·2

Discussion.

The mineral balances as tabulated for the 1st and 2nd weeks, when the unsupplemented basal ration was fed indicate that all the animals behaved similarly. They were in slightly negative Ca and slightly positive P balances. Negligible quantities of phosphorus, but fair proportions of calcium were eliminated in the urine.

As already stated the mineral balance of only one animal in each group has been reported, the same animal being killed at the end of the experiment for the chemical and histological study of the bones.

Group I. Bovines 5154 *and* 5163.

The average daily mineral intake was 22·91 gm. Ca and 10·49 gm. P, of which bovine 5154 retained 9·69 gm. Ca and 6·58 gm. P, being 42·3 and 62·7 per cent. respectively.

Both animals grew normally and maintained an excellent condition throughout the experiment, the inorganic phosphorus, calcium and phosphatase contents of the blood remaining normal.

On the unsupplemented basal ration these animals already showed a slightly positive balance. Furthermore the phosphorus of di-sodium phosphate has been shown to be in the vicinity of 100 per cent. available, and as 7 gm. P in the form of di-sodium phosphate was added to the ration, 62 per cent, retention of phosphorus seems a likely value on the combined P intake.

Slightly less Ca than anticipated was retained, the ratio of Ca : P retained being 1·6 : 1 instead of the usually accepted 2 : 1 ratio. Although it is impossible to state from the balance trials that these animals received enough P for their requirements, their weight increase, inorganic P content of the blood as well as earlier work on P requirement do suggest that such was the case. The ratio in which the P when compared with Ca was present also favoured maximum absorption.

Each of the four bones analysed of bovine 5154 contained the lowest proportion of water and fat and the highest proportion of dry-fat-free bone. The ash content of the dry-fat-free bones suggest good calcification. The ash content of the green bones varied from 34·4 per cent. for the humerus to 44·4 per cent. for the metacarpus.

The metacarpus of Bovine 5154 registered a breaking pressure of 2280 lb. per square inch over a six inch span, which is higher than that obtained in all the other groups except group V.

Group II. Bovines 5155 and 5157.

Average daily mineral intake: 22·61 gm. Ca and 3·44 gm. P. While the calcium intake was kept at the same level as that of the previous group, viz. 22·9 gm. Ca per day, the phosphorus level was reduced to 3·44 gm. P per day. This P was contained entirely in the basal ration. Shortly after the commencement of the experiment, the animals of group II began losing their appetite and its was necessary to reduce the samp of the ration from 4 Kg. to 2 Kg. per day and eventually increase it again to 3 Kg.

During the first couple of months while these animals consumed their full rations, they gained normally in weight, but on the reduced rations they became thin and lost weight, finally weighing about 300 lb. less than those of group I.

In the 8th month of the experiment the exceedingly low inorganic phosphorus value of 1·7 mgm. per 100 c.c. blood was obtained, showing that the animals of group II were suffering from severe aphosphorosis. At the beginning of the 18th month the phosphatase content of the blood was 9·0 units (Bodansky) which is indicative of disturbed calcium-phosphorus metabolism. Commencing on the day after they had been bled for the determination of the above constituents, the animals of this group were given ·53 gm. P as di-sodium phosphate daily for a period of three months. The mineral balance was determined a fortnight after the addition of the phosphorus supplement.

The addition of ·53 gm. P to the ration of this group favourably influenced the inorganic phosphorus and phosphatase contents of the blood. When the supplementation was discontinued at the beginning of the 21st month, this beneficial effect was lost. The amount of phosphorus supplemented was, however, insufficient to relieve the phosphorus deficiency and restore the blood constituents to normal.

Bovine 5157 retained 4·38 gm. Ca and ·35 gm. P per day, being 19·4 per cent. and 10·2 per cent of the intakes respectively. The exceedingly low phosphorus content of the ration adversely affected the retention of the calcium, which dropped to less than half that of the previous group on the same level of calcium intake.

It seems remarkable that so little P was retained in the body over the comparatively long period of the experiment as reference to Table XLII in the appendix will indicate. What new bone was formed must have derived its Ca and P almost entirely by resorption from the skeleton and substituting inferior bone. The bone analysis bears out this contention.

The green bones of group II were considerably lighter in weight, the metacarpus and the rib being about ⅔ of that of the corresponding bones of group I. The percentage of dry-fat-free bone was from about 10-13 per cent less and the specific gravities slightly less than those of group I. The metacarpus registered a breaking strength of 1,800 lb. per square inch as against 2280 lb. per square inch for the same bone of group I. The percentage ash in both the dry-fat-free bones and the green bones was low.

The ration did not supply sufficient phosphorus and only a negligible quantity of that present could be utilized, whereas a considerable amount of calcium was retained. The results of the bone analyses illustrated clearly the abnormal calcification. Although the ash content was very low its Ca : P ratio remained normal.

The Ca : P ratio of the ration was too high and the level of phosphorus intake too low to permit of sufficient minerals being retained in the correct proportion for normal bone calcification and general health of the animals.

Group III. Bovines 5158 *and* 5159.

Average daily mineral intake: 43·3 gm. Ca and 3·03 gm. P.

With the phosphorus content of the ration at the same deficient level and the calcium content double that of group II, the debility and loss of appetite of the animals were greatly aggravated. Shortly after the experimental ration had been given, these animals showed signs of severe anorexia; consequently the ration was reduced to 2 Kg. samp. Both animals showed stiffness and were in a very poor general condition. For the first couple of months, while on the basal ration, they gained in weight. In the third month prior to the feeding of the experimental ration, bovine 5158 weighed 630 lb. which was also, in the 22nd month its final weight, being 160 lb. less than the weight of bovines 5157 of the previous group.

The inorganic phosphorus and phosphatase contents of the blood of group III attained the same abnormally low value as that of the previous group. The addition of 1·07 gm. P in the form of di-sodium phosphate from the 18th to the 21st month, resulted in a higher inorganic phosphorus and a lower phosphatase content than were obtained by the addition ·53 gm. P to the ration of group II. These results suggest better calcification during that period in group III than in group II, which is confirmed by more favourable retention of P during that period by the former group than by the latter.

The 1·07 gm. P supplemented had no lasting effect, for when the supplement was discontinued, these blood constituents returned to their former values.

The average daily mineral retention of bovine 5158 was 10·58 gm. Ca and ·68 gm. P, being 24·4 per cent and 22·4 per cent. of the intake respectively. As in group II a high proportion of calcium was eliminated in the urine. In view of the fact that the Ca : P ratio of bone ash remained practically constant, the data obtained do not permit of explaining what happened to the relatively large

amount of calcium which was retained by the animal. The animals of group III drank almost double the amount of water inbibed by the other groups.

The form in which the phosphorus appears in the ration is undoubtedly of great importance for, during the three months from the beginning of the 18th to the beginning of the 21st month, when 1·07 gm. P in the form of di-sodium phosphate was added to the ration, the average daily retention was 1·27 gm. P, whilst during the 21st month the retention was ·22 gm. P, indicating that the entire amount of phosphorus added must have been retained by the animal. The phosphorus intake was admittedly lower during the 21st month than during the three previous months, but higher than during the 17th month although the retention was the same.

The average daily retention of phosphorus for the entire period was slightly better in this group than in group II. The retention during the three months of supplementary phosphate feeding greatly contributed to the average retentions of groups II and III.

The green bones of Bovine 5158 weighed less than those of any of the other groups but the percentages of dry-fat-free bone and the specific gravities were slightly higher than those of Bovine 5157 of group II. The breaking strength of the metacarpus was 1,200 lb. per square inch, being the lowest figure obtained in the experiment. For each of the bones the percentage ash in the green bone was higher in this group than in group II, but considerably lower than in group I. This may be ascribed to the fact that group III retained slightly more phosphorus.

Bovine 5158 of group II would with little doubt have died before the end of the experiment if the supplements of phosphorus had not been given. This addition, though small, undoubtedly influenced the figures obtained in the analyses.

Group IV. *Bovines* 5160 *and* 5161.

Average daily mineral intake. 4·23 gm. Ca and 3·44 gm. P.

The rations of these animals were not supplemented with minerals; therefore both the calcium and the phosphorus were at the lowest levels. The Ca : P ratio of the ration was 1·2 : 1, which could probably still be regarded as normal.

Although these animals did at times leave some of their food over, they never showed loss of appetite to such an extent that it was necessary to reduce their rations for more than a week at a time. They gained in weight almost to the same extent as those of the normal group, viz. I. If, however, all the groups had been fed *ad lib*, it is doubtful whether this group would have consumed more whereas group I would certainly have eaten more and consequently have gained more in weight.

Bovine 5160 showed an average daily retention of ·76 gm. P being 22 per cent. of the intake. The calcium balance oscillated between slightly negative and slightly positive values.

It is surprising that although the animals retained small amounts of phosphorus, the calcium which was well absorbed was eliminated in the urine.

The inorganic blood phosphorus values were at the same low level as in the previous groups, decreasing finally to 1·4 mgm. per 100 c.c. blood. The phosphatase content reached a high figure of 16·2 units (Bodansky) per 100 c.c. blood. The final values for these blood constituents showed a more severe degree of mineral deficiency than those for groups II and III.

The blood calcium values were uneffected, remaining normal at almost 10 mgm. per 100 c.c. blood.

The green rib of group IV contained 25·3 per cent. ash as against 38·5 per cent. for that of group I. Therefore 100 gm. green rib of the latter group could lose 13·2 gm. i.e. 34·3 per cent. of its ash before attaining the same mineral content as that of group IV.

The dry-fat-free bone and percentage ash suggest poor calcification which is confirmed by the low breaking strength of the metacarpus.

Group V. Bovines 5153 and 5167.

Daily average mineral intake: 45 gm. Ca and 10·68 gm. P.

The calcium content of this ration was equal to that of group III but twice that of group I, while the phosphorus content was equal to that of group I. The Ca : P ratio was 4·21 : 1, being almost double that of the normal group.

The average daily retention of calcium was 14·76 gm. and of phosphorus 5·31 gm. being 32·8 per cent. and 49·8 per cent. of the intake respectively. When a comparison is made with group I, it appears that increased calcium had a disadvantageous effect upon the phosphorus retention while increased phosphorus had a beneficial effect upon the retention of calcium.

Although the Ca : P ratio of the ration of this group was slightly lower than that of group II, it was at a higher level of mineral intake, which allowed sufficient minerals to be retained for the purpose of proper bone formation, the excess being discarded in the excreta.

It is noteworthy that towards the end of the experiment, the phosphorus content of the urine of group V was about 20 times higher than normal, whereas negligible amounts of phosphorus were contained in the urine of group I which was on the same level of phosphorus intake. The calcium content of the urine of group V remained normal. It appears that this abnormal elimination of phosphorus in the urine is associated with the excess of calcium in the ration.

The inorganic phosphorus and the phosphatase content of the blood remained normal.

The breaking strength of the metacarpus indicates well calcified bone. As a matter of fact the value obtained over a six inch span was about 400 lb. higher than that of the metacarpus of the animal killed in group I, receiving enough Ca and P.

The values for the percentage dry-fat-free bones, their ash contents, and the ash contents of the green bones were slightly lower than those of group I.

These animals consumed their full rations throughout the experiment and gained normally in weight.

Group VI. *Bovines* 5147 *and* 5149.

Average daily mineral intake 4·14 gm. Ca and 10·68 gm. P.

The phosphorus content of the ration of group VI was equivalent to that of the normal group, being 10·68 gm. P while the calcium content was reduced to 4·14 gm. Ca, which was the same as that of group IV.

The average daily retention of 4·29 gm. P, i.e. 40·2 per cent. of the intake was lower than that retained by either group I or group V, which were on the same level of phosphorus intake but higher Ca. Bovine 5147 retained 1·12 gm. Ca per day, i.e. 27 per cent of the intake. A high proportion of phosphorus was eliminated in the urine. At times this figure was about equal to that excreted in the faeces and more than double that retained by the animal.

These animals ate well and gained normally in weight, the inorganic phosphorus, phosphatase and calcium contents of the blood remaining normal. There was no indication during life of a deficiency of either mineral.

The results of the bone analyses, however, showed signs of abnormal bone formation. The percentage dry-fat-free bone and ash content of the femur, humerus and metacarpus were only slightly lower than in those of groups I to V. The rib, however, showed more marked differences.

If the data obtained in this group be compared with those of the phosphorus deficient groups the conclusions appear justified that low phosphorus in the ration of stock when sufficient calcium is present is more detrimental than when low calcium with sufficient phosphorus is given.

A probable explanation for this finding is that their calcium requirement is lower than their phosphorus requirement.

General.

Three groups, namely, groups I, V and VI were given a ration containing the same amount of phosphorus but with the Ca : P ratios of about 2 : 1, 4 : 1 and 0·26 : 1 respectively. These represented the optimal, the high and the low ratios of this experiment.

Group I showed the highest percentage retention of both calcium and phosphorus. In group V there was a depression of the phosphorus retention and an increased calcium retention.

It would appear that the deficiency of calcium in group VI, receiving a phosphorus adequate ration, had a more detrimental effect upon the total retention of phosphorus, than excess of calcium as in group V.

Compared with group IV, where both minerals were deficient, the balance figures of group VI suggest that in digestion excess of phosphorus over calcium increases the solubility of the calcium compounds and renders absorption of calcium easier.

The feeding of phosphorus adequate rations containing sufficient and excess calcium (groups I and V respectively) resulted in a better calcium retention than when the rations were phosphorus deficient (groups II and III).

In the three phosphorus deficient groups, namely II, III and IV, the average daily phosphorus retentions of groups III and IV were about the same, that of group II being slightly less. But as the animals of groups II and III were given a phosphate supplement for a period of three months, they are not quite comparable with group IV. If the balance figures for the three months of added phosphate feeding be omitted from the calculations it is found that group IV retained slightly more phosphorus than either groups II or III, between which there was no difference.

From these experiments it can however not be claimed that a wide Ca : P ratio in a phosphorus deficient ration caused a depression of the phosphorus retention as the retention of group II was lower than that of group III.

The blood analyses indicate that in the groups receiving adequate phosphorus, the inorganic phosphorus and the phosphatase content remained normal. If the phosphatase content of the blood is affected by a deficiency of calcium, then the degree of calcium deficiency, as experienced by group VI, was not acute enough to affect a rise in the phosphatase content of the blood.

The blood calcium values remained normal in all the groups, irrespective of the Ca : P ratio or the mineral content of the ration. As the rations of these animals contained sufficient vitamin D such values were anticipated.

A deficiency of phosphorus in the ration resulted in a loss of appetite. This condition was slighty noticeable in group IV fed on a ration with a normal Ca : P ratio, but became more pronounced as the Ca : P ratio was increased, those animals (group III) fed on the ration with a Ca : P ratio of 14·3 : 1 eating the least. When adequate phosphorus was fed, with either a deficiency of calcium (group VI) or excess calcium (group V) the abnormal Ca : P ratio of the ration caused no noticeable signs of anorexia.

It would seem therefore that the loss of appetite is associated with abnormal Ca : P metabolism rather than with a specific factor such as a low P or low Ca or abnormal ratio, which obviously might be responsible for the abnormal metabolism as stated by Theiler, du Toit and Malan (1937).

The green weights and measurements of the bones examined in groups II, III, IV and VI fed on rations deficient in either or both calcium and phosphorus, were smaller than those of groups I and V whose rations were adequately supplied with both elements, but as the green weight is also dependent upon the size of the bone—an inherited factor—it is obviously not as true a criterion as percentage ash for instance.

The determination of the percentage ash in the green bones affords an excellent index of the degree of calcification. Either calcium or phosphorus whichever was retained in lesser quantity, limits the degree of calcification. Thus, for example, a diet adequate in calcium but poor in phosphorus leads to a state of minimal phosphorus retention, and in such a case, a superfluity of calcium will not help to raise the total percentage of ash in the bones above that determined by the lesser quantity of phosphorus retained.

From the data obtained the Ca : P ratio of the ration appears to be important for normal growth and development when the ration was deficient in either or both minerals, but was without appreciable influence in the case of one abnormal ratio where phosphorus was present in an adequate quantity (group V).

In a paper by Theiler, du Toit and Malan (1937) full details of weights, blood analyses, clinical symptoms, histological examination of the bones and the development of osteodystrophic diseases are given and discussed.

Summarizing the results of the experiment described one is led to conclude that 22·9 gm. Ca and 10·5 gm. P, of which respectively 9·69 and 6·58 gm. were retained provided sufficient Ca and P for normal growth.

The retention of P on the same level of intake was affected by the amount of Ca present as indicated in the following table.

Ca intake.	*P intake.*	*Ca retained.*	*P retained.*
22·9	10·49	9·7	6·6
45·0	10·49	14·7	5·3
4·14	10·49	1·1	4·3

The Ca retention is decreased or increased by respectively decreasing or increasing the amount of the Ca ingested. When the P intake was reduced to approximately 3 gm. the best retention of P was shown when the Ca intake was proportionately decreased.

It appears that the main factor which determines retention of Ca or P is the level of intake of the mineral in question apart from the ratio in which it is associated with the remaining mineral in the food.

SUMMARY.

1. The availability of the P in several commercial phosphates was determined in balance experiments with bovines. The availability was measured in terms of the retention of P. High

retention was favoured by giving the phosphates at a level, viz. 6 gm. P, well below that of the P requirement of the class of animal used. The ratio of Ca : P was kept constant for all phosphates at approximately 2:1.

2. Di-sodium phosphate appeared to be slightly more available at the given level of P intake than either di-calcium phosphate or bonemeal, between whose retentions there was no significant difference. Taking all the results obtained into consideration it is doubtful whether animals when fed phosphates well below their requirements will retain more P when it is given as di-sodium phosphate than as the relatively insoluble di-calcium phosphate or bonemeal. In the experiments reported the best retention of P in di-sodium phosphate was 100 per cent. and that of di-calcium phosphate and bonemeal 97 and 94 per cent. respectively.

3. The retention of Ca which was supplied as $CaCO_3$ when insufficient was present in the phosphatic supplement and the basal ration varied from 65-100 per cent. irrespective of the supplement fed.

4. The remarkably high percentages of P retained when the supplement was fed well below the requirements of the animals were reduced by about 10 per cent. at approximately the optimal level of P intake.

5. Long term metabolism experiments were conducted with growing bovines fed on rations at different levels of mineral intake but with a constant optimal Ca : P ratio. At the end of the experiment these animals were killed and the bones used for chemical and histological studies.

6. Rations containing 8·5 gm. P and 16·4 gm. Ca i.e. 19·5 gm. P_2O_5 and 23 gm. CaO were found to be adequate for growing steers, whereas 5·8 gm. P and 12 gm. Ca and less were insufficient. Inorganic blood phosphorus, mineral balances and bone analyses were taken as criteria.

7. A determination of the percentage ash in the green bones afforded an excellent index of the degree of calcification. A close correlation was noted between the above index and the breaking strength of the metacarpus for the animals of the same age.

8. Data are further recorded of long term metabolism experiments conducted with growing heifers fed a ration containing different levels of P and Ca and hence varying Ca : P ratios.

9. 23 gm. Ca and 10·5 gm. P permitting an average daily retention of about 9·7 gm. Ca and 6·6 gm. P were found to be adequate for growing heifers, the conclusions being based on the mineral balances, and blood and bone analyses. The histological findings of the bones of the animals used in these experiments are discussed in detail by Theiler *et al* (1937).

10. The quantity of Ca or P present in the ration limited the retention of the remaining element. Varying the intakes of the calcium and the phosphorus from the optimum resulted in less efficient retention of both elements.

11. The quantities of Ca and P present in the ration apparently determined more than any other definable factor the quantities of these minerals retained in the body. A high intake of Ca or P or both is associated with high retention of that constituent.

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APPENDIX.

TABLE XXIV.

EXPERIMENT II.

Group I: Phosphorus Balance (daily average).

Week No.	Bovine 4712. Intake gm. P.	Outgo gm. P. Faeces.	Outgo gm. P. Urine.	Balance gm. P.	Month of Expt.	Bovine 3478. Intake gm. P.	Outgo gm. P. Faeces.	Outgo gm. P. Urine.	Balance gm. P.
1	4·51	4·24	·028	0·24	2	4·51	4·44	·031	0·04
2	4·51	4·88	·027	−0·40	3	4·51	4·48	·024	0·01
3	8·00	3·80	·025	4·12	4	8·00	4·51	·027	3·46
4	8·00	5·07	·036	2·89	5	8·00	4·42	·031	3·55
5	8·83	4·25	·043	4·54	6	8·83	5·00	·043	3·79
6	8·83	4·33	·050	4·45	8	8·83	4·10	·053	4·68
7	8·67	4·46	·061	4·15	9	8·67	3·76	·052	4·86
8	8·67	3·47	·075	5·12	10	8·67	3·96	·038	4·67
9	8·87	4·08	·060	4·73	11	8·87	3·48	·048	5·34
10	8·87	3·88	·071	4·92	12	8·87	3·15	·051	5·67
11	8·54	3·06	·047	5·43	13	8·54	3·36	·050	5·13
12	8·07	4·37	·049	3·65	15	—	—	—	—
13	7·73	4·37	·081	3·28	16	—	—	—	—
14	7·73	3·40	·080	4·25	17	—	—	—	—
15	8·17	4·50	·007	3·66	18	—	—	—	—
16	8·17	3·37	·013	4·79	20	—	—	—	—
17	8·14	3·82	·008	4·31	21	—	—	—	—
18	8·30	3·16	·008	5·13	24	—	—	—	—
19	8·14	4·50	·008	3·63	25	—	—	—	—
		Mean for weeks		3–19			Mean for weeks		3–11
	8·34			4·42		8·59			4·57

Table XXV.

EXPERIMENT II.

Group I: Calcium Balance (daily average).

Week No.	Bovine 4712. Intake gm. Ca.	Bovine 4712. Outgo gm. Ca. Faeces.	Bovine 4712. Outgo gm. Ca. Urine.	Bovine 4712. Balance gm. Ca.	Month of Expt.	Bovine 3478. Intake gm. Ca.	Bovine 3478. Outgo gm. Ca. Faeces.	Bovine 3478. Outgo gm. Ca. Urine.	Bovine 3478. Balance gm. Ca.
1	6·31	4·93	2·27	−0·89	2	6·30	3·62	3·12	−0·44
2	6·32	4·41	2·59	−0·68	3	6·36	3·23	2·71	0·42
3	15·47	7·48	1·24	6·75	4	15·40	6·12	1·04	8·24
4	15·49	8·00	1·71	5·78	5	15·52	6·40	0·98	8·14
5	17·44	7·21	1·43	8·80	6	17·48	8·42	1·22	7·84
6	17·49	9·07	1·74	6·68	8	17·51	8·10	1·66	7·75
7	17·48	9·07	1·48	6·93	9	17·51	7·43	1·44	8·64
8	16·07	8·21	1·34	6·52	10	16·17	8·14	1·23	6·80
9	15·87	7·93	1·46	6·48	11	15·86	7·64	1·64	6·58
10	15·88	8·14	1·35	6·39	12	15·80	9·00	1·28	5·52
11	15·84	8·07	1·59	6·18	13	15·88	8·93	1·76	5·19
12	15·84	8·13	1·03	6·68	15	—	—	—	—
13	16·11	6·46	1·75	7·90	16	—	—	—	—
14	15·78	8·50	1·66	5·62	17	—	—	—	—
15	16·46	9·07	1·71	5·68	18	—	—	—	—
16	16·31	8·74	1·74	5·83	20	—	—	—	—
17	16·88	11·88	1·00	4·00	21	—	—	—	—
18	16·88	9·00	1·14	6·74	24	—	—	—	—
19	16·88	10·42	1·36	5·10	25	—	—	—	—
		Mean for weeks		3–19			Mean for weeks		3–11
	16·36			6·36		16·35			7·19

Table XXVI.

EXPERIMENT II.

Group II: Phosphorus Balance (daily average).

Week No.	Bovine 3480. Intake gm. P.	Bovine 3480. Outgo gm. P. Faeces.	Bovine 3480. Outgo gm. P. Urine.	Bovine 3480. Balance gm. P.	Month of Expt.	Bovine 3465. Intake gm. P.	Bovine 3465. Outgo gm. P. Faeces.	Bovine 3465. Outgo gm. P. Urine.	Bovine 3465. Balance gm. P.
1	4·57	4·25	·033	0·29	2	4·50	4·01	·033	0·46
2	4·57	4·46	·031	0·08	3	4·50	4·38	·038	0·08
3	5·85	4·48	·034	1·34	4	5·78	4·20	·040	1·54
4	5·85	4·09	·035	1·72	5	5·78	4·65	·033	1·10
5	—	—	—	—	6	6·61	4·37	·046	2·19
6	—	—	—	—	8	6·61	3·98	·043	2·59
7	—	—	—	—	9	6·63	4·06	·042	2·53
8	—	—	—	—	10	6·63	4·39	·045	2·19
9	5·77	2·90	·039	2·83	11	6·65	4·55	·051	2·05
10	5·77	3·57	·053	2·15	12	6·65	3·69	·046	2·91
11	5·77	3·19	·044	2·54	13	—	—	—	—
12	5·86	3·22	·040	2·60	15	—	—	—	—
13	5·51	3·54	·042	1·93	16	—	—	—	—
14	5·51	3·37	·041	2·10	17	—	—	—	—
15	5·97	2·99	·044	2·94	18	—	—	—	—
16	5·97	2·61	·026	3·33	20	—	—	—	—
17	5·94	3·41	·048	2·48	21	—	—	—	—
18	6·10	3·61	·048	2·44	24	—	—	—	—
19	5·94	4·13	·057	1·75	25	—	—	—	—
		Mean for	weeks	3–19			Mean for	weeks	3–19
	5·83			2·47		6·42			2·14

Table XXVII.

EXPERIMENT II.

Group II: Calcium Balance (daily average).

Week No.	Bovine 3480. Intake gm. Ca.	Bovine 3480. Outgo gm. Ca. Faeces.	Bovine 3480. Outgo gm. Ca. Urine.	Bovine 3480. Balance gm. Ca.	Month of Expt.	Bovine 3465. Intake gm. Ca.	Bovine 3465. Outgo gm. Ca. Faeces.	Bovine 3465. Outgo gm. Ca. Urine.	Bovine 3465. Balance gm. Ca.
1	6·29	4·48	2·64	−0·83	2	6·32	4·52	1·98	−0·18
2	6·32	5 07	2·14	−0·89	3	6·30	3·78	2·45	0·07
3	11·45	7·59	0·94	3·02	4	11·65	6·90	1·18	3·57
4	11·53	7·08	0·93	3·52	5	11·50	8·64	1·22	1·64
5	—	—	—	—	6	13·63	7·53	·91	5·09
6	—	—	—	—	8	13·52	7 28	·93	5·31
7	—	—	—	—	9	13·49	6·90	·89	5·70
8	—	—	—	—	10	13 51	7·95	·84	4·72
9	11·87	6 64	0·74	4·49	11	11·90	7·93	·90	3·07
10	11·90	8·28	1·33	2·29	12	11·87	7·05	·85	3·97
11	11·88	7·19	0·76	3·93	13	—	—	—	—
12	11·33	7·28	1·48	2·57	15	—	—	—	—
13	11·76	6·85	1·28	3·63	16	—	—	—	—
14	11·77	5·28	2·12	4·37	17	—	—	—	—
15	12·51	7·59	1·81	3·11	18	—	—	—	—
16	12·43	7·90	1·28	3·25	20	—	—	—	—
17	12·90	6·33	1·71	4·86	21	—	—	—	—
18	12·87	6·64	1·50	4·73	24	—	—	—	—
19	12·88	7·14	1·86	3·88	25	—	—	—	—
		Mean for weeks		3–19			Mean for weeks		3–10
	12·09			3·67		12·62			4·13

Table XXVIII.

EXPERIMENT II.

Group III: Phosphorus Balance (daily average).

Week No.	Bovine 3464. Intake gm. P.	Outgo gm. P. Faeces.	Outgo gm. P. Urine.	Balance gm. P.	Month of Expt.	Bovine 3467. Intake gm. P.	Outgo gm. P. Faeces.	Outgo gm. P. Urine.	Balance gm. P.
1	4·56	3·72	·033	0·81	2	4·56	4·35	·029	0·18
2	4·56	4·33	·035	0·19	3	4·56	4·59	·034	-0·06
3	5·84	4·18	·037	1·62	4	5·84	4·11	·042	1·69
4	5·84	3·78	·031	2·03	5	5·84	4·33	·039	1·47
5	4·26	2·81	·036	1·41	6	5·79	3·20	·043	2·55
6	4·26	2·50	·037	1·72	8	5·79	3·37	·050	2·37
7	4·92	2·70	·045	2·17	9	5·79	3·75	·051	1·99
8	4·92	3·35	·053	1·52	10	5·79	3·21	·050	2·53
9	4·89	2·19	·049	2·64	11	5·68	2·67	·052	2·96
10	5·68	2·92	·050	2·71	12	5·68	3·41	·053	2·22
11	5·68	2·70	·050	2·93	13	5·68	2·62	·045	3·01
12	5·87	3·50	·034	2·34	15	5·87	3·94	·054	1·88
13	5·53	4·21	·045	1·27	16	5·53	4·62	·045	0·86
14	5·53	3·74	·031	1·76	17	5·97	3·96	·031	1·98
15	5·97	4·11	·048	1·81	18	5·97	3·04	·048	2·88
16	5·97	3·82	·052	2·10	20		—		—
17	5·94	4·27	·061	1·61	21		-		
18	6·10	3·43	·092	2·58	24	-	-		--
19	5·94	3·52	·048	2·37	25	--	—	--	—
		Mean for weeks		3–19			Mean for weeks		3–15
	5·48			2·03		5·79			2·18

Table XXIX.

EXPERIMENT II.

Group III: Calcium Balance (daily average).

Week No.	Bovine 3464. Intake gm. Ca.	Outgo gm. Ca. Faeces.	Outgo gm. Ca. Urine.	Balance gm. Ca.	Month of Expt.	Bovine 3467. Intake gm. Ca.	Outgo gm. Ca. Faeces.	Outgo gm. Ca. Urine.	Balance gm. Ca.
1	6·30	4·19	2·48	−0·37	2	6·28	4·62	1·98	−0·32
2	6·30	4·18	3·43	−0·31	3	6·34	4·47	2·20	−0·33
3	24·41	18·35	2·35	3·71	4	24·38	18·09	3·24	3·05
4	24·38	15·85	2·38	6·15	5	24·38	17·17	2·53	4·68
5	23·04	13·78	2·88	6·38	6	24·94	18·64	3·50	2·80
6	22·33	16·42	1·56	4·35	8	24·91	16·14	3·70	5·07
7	23·35	16·78	1·73	4·84	9	24·96	14·57	3·04	7·35
8	23·24	14·42	1·74	7·08	10	24·95	18·49	3·62	2·84
9	22·83	16·57	1·28	4·98	11	23·31	13·07	3·57	6·67
10	22·83	16·14	2·96	3·73	12	23·26	17·93	3·74	1·60
11	22·81	17·28	3·33	2·20	13	23·28	14·14	2·59	6·55
12	24·72	19·21	2·77	2·74	15	24·73	19·14	3·60	1·99
13	24·54	17·46	3·00	3·08	16	24·56	17·85	3·44	3·27
14	24·53	16·71	3·14	4·68	17	24·55	19·64	3·51	1·40
15	25·80	18·72	1·44	5·04	18	25·28	15·10	2·41	7·77
16	25·23	16·04	1·94	7·25	20	—	—	—	—
17	25·73	15·21	2·28	8·24	21	—	—	—	—
18	25·71	17·21	2·86	5·64	24	—	—	—	—
19	25·71	16·92	2·43	6·36	25	—	—	—	—
		Mean for weeks		3–19			Mean for weeks		3–15
	24·09			5·09		24·42			4·23

Table XXX.

EXPERIMENT II.

Group IV: Phosphorus Balance (daily average).

Week No.	Bovine 3454. Intake gm. P.	Bovine 3454. Outgo gm. P. Faeces.	Bovine 3454. Outgo gm. P. Urine.	Bovine 3454. Balance gm. P.	Month of Expt.	Bovine 3456. Intake gm. P.	Bovine 3456. Outgo gm. P. Faeces.	Bovine 3456. Outgo gm. P. Urine.	Bovine 3456. Balance gm. P.
1	4·54	4·03	·031	0·48	2	4·54	4·20	·031	0·31
2	4·54	3·83	·030	0·68	3	4·54	4·47	·027	0·04
3	4·54	4·24	·037	0·26	4	3·47	4·61	·028	−1·17
4	4·54	4·15	·031	0·36	5	3·79	4·37	·032	−0·61
5	5·37	3·58	·032	1·76	6	4·16	3·47	·033	0·66
6	4·38	2·39	·034	1·96	8	2·20	1·53	·033	0·64
7	5·15	3·45	·038	1·66	9	3·44	1·77	·036	1·63
8	—	—	—	—	10	2·73	1·75	·037	0·94
9	—	—	—	—	11	4·89	2·27	·038	2·58
10	—	—	—	—	12	4·85	2·32	·040	2·49
11	—	—	—	—	13	4·48	2·25	·032	2·20
12	—	—	—	—	15	4·23	2·19	·019	2·02
13	—	—	—	—	16	2·93	2·50	·055	0·37
14	—	—	—	—	17	2·56	1·96	·039	0·56
15	—	—	—	—	18	3·76	2·36	·031	1·37
16	—	—	—	—	20	3·28	2·16	·031	1·09
17	—	—	—	—	21	3·71	2·78	·052	0·88
18	—	—	—	—	24	3·87	2·26	·061	1·55
19	—	—	—	—	25	3·50	2·46	·048	0·99
		Mean for weeks		3–7			Mean for weeks		3–19
	4·80			1·20		3·64			1·07

Table XXXI.

EXPERIMENT II.

Group IV: Calcium Balance (daily average).

Week No.	Bovine 3454. Intake gm. Ca.	Bovine 3454. Outgo gm. Ca. Faeces.	Bovine 3454. Outgo gm. Ca. Urine.	Bovine 3454. Balance gm. Ca.	Month of Expt.	Bovine 3456. Intake gm. Ca.	Bovine 3456. Outgo gm. Ca. Faeces.	Bovine 3456. Outgo gm. Ca. Urine.	Bovine 3456. Balance gm. Ca.
1	6·30	4 53	2·64	−0·87	2	6·32	3·89	2 43	—
2	6 30	4 00	2·08	0·22	3	6·33	3·86	2·66	−0·19
3	8 69	5 01	1·90	1 78	4	6·30	5·46	2·23	1 39
4	8·70	5·93	1·54	1·23	5	6·98	6 46	2·43	1 91
5	10·67	4·53	1·54	4·60	6	8·54	4 53	2 28	1·73
6	8·38	3·61	2·24	2·53	8	7·67	2 21	2 43	3 03
7	8·37	3 64	2·41	2·32	9	8 66	3·15	2 64	2 87
8		—	–	–	10	8·31	3 75	2 53	2·03
9	-	—		—	11	7·99	3·52	2·53	1 94
10	—	—		–	12	8·73	3 38	2 88	2·47
11	—			—	13	8·71	3·53	2 53	2 65
12	--		--		15	7·95	3 25	2 07	2 63
13		-			16	6·23	3·93	2 06	0 24
14				-	17	3·72	1·34	2 24	0·14
15		--		-	18	9·00	3·51	4·26	1·23
16					20	7·55	3·02	2 32	2·21
17		—		--	21	9·37	4 86	1·36	3·15
18	---		—	—	24	9·34	3 88	1 50	3·96
19	—		—	—	25	9·97	5·31	1·43	3·23
		Mean for	weeks	3-7			Mean for	weeks	3-19
	8 96			2·49		7·94			1·78

Table XXXII.

EXPERIMENT II.

Group V: Phosphorus Balance (daily average).

Week No.	Bovine 5430. Intake gm. P.	Outgo gm. P. Faeces.	Outgo gm. P. Urine.	Balance gm. P.	Month of Expt.	Bovine 5431. Intake gm. P.	Outgo gm. P. Faeces.	Outgo gm. P. Urine.	Balance gm. P.
1	1·01	1·37	·018	−0·38	15	1·01	1·50	·022	−0·51
2	1·01	1·52	·026	−0 54	16	1·01	1·44	·031	- 0·46
3	1·29	2 01	·031	0·75	17	1·29	2·00	·031	−0·74
4	1·29	1·99	·035	0 74	18	1·29	2 03	·026	−0·77
5	1·71	2·36	·035	−0·69	19	1·71	2·27	·039	−0·60
6	1·71	1 82	·026	- 0·14	20	1·71	1·72	·035	−0·05
7	1·71	2·14	018	−0·45	21	1·71	1 61	·035	0·06
8	1·71	1·82	·018	- 0·13	22	1 71	1·39	·031	0·29
9	1·71	1·62	·044	0 05	23	1·71	1·44	031	0·24
10	1·71	1·38	044	0 29	24	1·71	1 75	·026	0·07
11	1 98	1·48	·031	0 47	25	1 98	1·47	·048	0·46
12	1·98	1·68	038	0 26	26	1·98	1 35	·038	0·59
		Mean.					Mean.		
	1·68			·11		1·68			— 05

Table XXXIII.

EXPERIMENT II.

Group V: Calcium Balance (daily average).

Week No.	Bovine 5430. Intake gm. Ca.	Outgo gm. Ca. Faeces.	Outgo gm. Ca. Urine.	Balance gm. Ca.	Month of Expt.	Bovine 5431. Intake gm. Ca.	Outgo gm. Ca. Faeces.	Outgo gm. Ca. Urine.	Balance gm. Ca.
1	2·14	3 05	0·80	−1·71	15	2·16	2·98	0·84	−1·66
2	2·15	2·47	1·03	−1·35	16	2·17	2 51	0·80	−1·14
3	2·17	2·44	1·87	−2·14	17	2·16	1·36	2·21	−1·41
4	2·18	1·47	2·68	−1·97	18	2·19	1·22	3·15	−2·18
5	2·67	1·85	2·30	−1·48	19	2 68	·85	3·00	−1·17
6	2·71	2·83	1·88	−2·00	20	2·70	·89	2·91	−1·10
7	2·81	2·22	1·43	- 0·84	21	2·78	1·16	1·29	0·33
8	2 78	1·37	0·70	0·71	22	2·80	1·23	1·41	0·16
9	2·80	1·45	0·86	0·49	23	2·81	1·32	1·07	0 42
10	2·83	1·04	1·03	0·76	24	2·82	1·89	0·75	0·18
11	3·00	1·25	1·21	0·54	25	3·03	1·43	·87	0·73
12	3·02	1·36	1·09	0 57	26	3·01	1·52	·91	0·58
		Mean for	weeks	3–12			Mean for	weeks	3–12
	2·70			−0·54		2·70			·0·35

TABLE XXXIV.

EXPERIMENT II.

Weights of Animals in lb.

Date.	Month of Experiment.	Group I.		Group II.		Group III.		Group IV.		Group V.	
		4712.	3478.	3480.	3465.	3464.	3467.	3454.	3456.	5430.	5431.
1933.											
June	1	821	800	812	767	798	818	756	836	—	—
July	2	841	810	831	790	796	831	779	857	—	—
August	3	880	860	863	814	834	854	814	855	—	—
September	4	890	865	873	827	851	860	820	860	—	—
October	5	861	861	863	816	832	845	797	836	—	—
November	6	894	887	883	839	839	845	833	839	—	—
December	7	900	886	819	844	820	844	855	838	—	—
1934.											
January	8	906	914	824	850	815	871	863	848	—	—
February	9	934	934	850	865	805	865	883	855	—	—
								Died			
March	10	949	983	872	874	816	880	—	849	—	—
April	11	975	1,000	885	888	830	895	—	850	—	—
May	12	995	1,004	875	890	826	882	—	851	—	—
June	13	1,020	1,036	875	Died	853	890	—	875	—	—
July	14	1,014	Died	910	—	892	896	—	869	450	400
August	15	1,000	—	935	—	920	915	—	880	—	—
September	16	1,040	—	925	—	915	945	—	865	470	440
October	17	1,065	—	950	—	925	1,000	—	845	490	460
November	18	1,060	—	940	—	925	Died	—	820	510	500
December	19	—	—	—	—	—	—	—	—	500	508
1935.											
January	20	1,085	—	960	—	940	—	—	860	495	500
February	21	1,100	—	1,000	—	965	—	—	840	520	520
March	22	1,125	—	970	—	1,000	—	—	875	560	540
April	23	—	—	—	—	—	—	—	—	580	550
May	24	1,170	—	1,055	—	1,050	—	—	895	615	600
June	25	1,215	—	1,100	—	1,080	—	—	890	615	610
July	26	1,265	—	1,130	—	1,105	—	—	885	625	620

TABLE XXXV.

EXPERIMENT III.

Inorganic Blood Phosphorus in mgm. per 100 c.c. Blood.

Group No.	Bovine.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.
I........................	5154	5·4	5·0	5·2	5·0	4·6	5·9	6·5	—	6·1	5·9	7·7	5·8	7·5	5·2	6·7
	5163	5·1	4·9	4·4	4·6	5·1	5·5	4·5	—	5·2	6·7	5·1	4·5	5·3	4·7	4·8
II........................	5155	1·7	1·5	1·6	1·9	1·8	2·2	1·8	—	2·3	2·1	2·4	3·0	2·9	2·9	2·4
	5157	1·7	1·7	1·6	2·3	1·8	1·5	1·5	—	3·0	2·5	2·5	3·0	2·3	3·0	2·0
III........................	5158	1·9	2·2	2·0	1·8	1·7	3·4	2·4	—	3·0	2·6	2·4	4·6	4·1	4·3	2·4
	5159	1·6	1·5	1·9	2·3	2·0	1·6	1·4	—	Died	—	—	—	—	—	—
VI........................	5160	1·9	2·0	2·1	2·3	1·7	1·5	1·7	—	2·4	3·0	1·6	1·6	1·3	1·3	1·4
	5161	1·6	1·6	2·0	1·8	1·6	1·9	1·3	—	3·0	3·4	Died	—	—	—	—
V........................	5153	5·8	5·7	5·3	6·1	7·3	5·8	5·6	—	7·8	7·3	5·0	5·0	5·3	5·0	5·0
	5167	6·0	6·0	7·7	5·7	6·4	5·9	4·6	—	7·7	7·1	5·4	5·7	5·2	5·7	5·2
VI........................	5147	5·2	4·9	4·8	3·8	4·8	4·8	5·8	—	5·5	6·8	4·0	4·2	5·2	5·4	4·4
	5149	5·1	5·0	5·8	4·6	5·7	5·4	5·9	—	6·6	6·4	5·1	4·6	5·9	4·1	4·9

Table XXXVI.

EXPERIMENT III.

Blood Calcium given Monthly in mgm. per 100 c.c. Blood.

Group No.	Bovine No.	Month of Experiment.														
		8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.
I.	5154	10·6	9·8	11·0	10·3	10·2	9·9	10·3	—	10·0	10·1	8·5	11·2	9·5	8·7	8·7
	5163	11·5	9·8	10·0	11·1	10·8	10·5	10·2	—	10·5	11·0	10·0	8·2	9·2	8·6	9·8
II.	5155	11·6	10·6	10·6	10·2	10·6	11·3	10·5	—	10·0	11·1	10·0	9·8	8·9	9·8	9·8
	5157	11·2	9·8	11·0	10·5	10·5	11·5	11·1	—	10·0	11·3	9·8	9·3	8·7	9·4	9·6
III.	5158	11·8	10·0	10·6	11·1	10·0	11·0	10·3	—	11·0	11·1	9·6	8·9	8·4	9·4	10·0
	5159	10·7	9·6	9·6	11·2	10·8	11·4	10·1	—	—	—	—	—	—	—	—
IV.	5160	10·7	10·0	10·0	10·6	11·0	11·5	10·6	—	10·3	11·2	9·8	8·8	10·8	9·8	9·5
	5161	10·2	11·2	11·0	11·4	10·5	11·3	11·0	—	10·1	10·7	—	—	—	—	—
V.	5163	11·0	9·8	9·6	10·4	10·2	10·6	10·0	—	9·8	10·2	9·2	8·7	8·2	8·7	8·5
	5167	10·9	10·6	11·0	10·6	10·4	11·0	10·6	—	10·6	10·6	10·2	8·7	9·7	8·6	9·1
VI.	5147	10·0	10·4	9·8	10·5	10·8	10·6	10·6	—	10·1	10·8	10·3	10·0	9·1	8·5	9·8
	5149	10·8	10·2	10·2	10·1	10·0	10·3	10·5	—	10·0	10·4	9·8	8·4	8·7	8·4	8·6

Table XXXVII.

EXPERIMENT III.

Phosphatase Content of Blood in Bodansky Units.

Group.	Bovine No.	Month of Experiment. 18.	19.	20.	21.	22.
I............	5154	3·5	3·3	4·6	4·4	3·9
	5163	5·2	1·6	5·0	5·1	5·3
II..........	5155	8·6	6·4	7·1	8·5	9·0
	5157	9·4	7·5	9·6	8·2	9·8
III..........	5158	9·4	3·8	5·5	7·7	8·9
IV............	5160	13·9	9·9	16·2	11·7	12·1
	5161	8·5	—	—	—	—
V............	5153	5·3	3·0	4·1	5·4	4·9
	5167	3·6	2·8	4·4	3·9	3·8
VI............	5147	2·8	·5	5·2	4·0	5·2
	5149	4·9	2·5	4·2	4·1	2·6

Table XXXIX.

EXPERIMENT III.

Weights in lb.

Month of Expt.	I. 5154	I. 5163	II. 5155	II. 5157	III. 5158	III. 5159	IV. 5160	IV. 5161	V. 5163	V. 5167	VI. 5147	VI. 5149
1	640	600	580	570	570	620	650	600	670	510	580	573
2	685	600	600	625	600	655	630	540	715	515	625	605
3	655	630	630	630	630	685	700	605	720	550	640	585
4	675	670	640	635	625	700	700	625	775	575	650	600
5	635	710	660	669	625	740	715	690	780	570	670	625
6	735	730	690	710	660	740	755	695	800	580	720	670
7	765	740	705	710	650	745	765	700	800	620	700	685
8	780	790	735	720	665	770	825	745	855	670	760	725
9	840	800	740	750	650	760	825	760	885	715	800	760
10	850	805	715	750	600	745	840	770	905	700	755	755
11	890	855	710	765	600	725	865	800	925	750	770	780
12	915	840	710	735	575	725	890	825	950	785	805	805
13	930	980	700	770	560	Died	885	845	985	800	865	810
14	935	900	715	765	580	—	895	835	1,020	835	900	835
15	955	920	725	775	585	—	900	850	1,025	855	885	870
16	1,000	955	725	750	600	—	925	900	1,035	910	925	865
17	1,045	955	705	785	595	—	965	925	1,080	915	945	900
18	1,020	995	685	745	610	—	970	920	1,065	945	945	930
19	1,060	990	725	765	615	—	1,000	Died	1,100	960	965	940
20	1,075	1,070	705	780	625	—	1,010	—	1,100	980	960	920
21	1,100	1,050	740	770	620	—	1,040	—	1,160	1,010	1,000	1,000
22	1,100	1,035	735	790	630	—	1,020	—	1,155	1,030	1,020	985

TABLE XXXVIII.

EXPERIMENT III.

Bone Analysis Bovines.

Bovine.	Group.	Green Weight.	Green Bone.				Breaking Strength per sq. in. 6 in. span.	Dry fat-free bone.			Percentage Ash of Green weight.
			Percentage Water.	Percentage Fat.	Percentage Dry fat free.	Sp. gr.		Percentage Ash.	Percentage Ca.	Percentage P.	
					FEMUR.						
5154..........	I.......	2098	12·05	34·85	53·1	1·296	2850	66·5	38·7	17·7	35·4
5157..........	II.......	1794	15·84	43·62	40·54	1·142	2650	61·8	39·4	17·1	25·1
5158..........	III.......	1622	14·68	41·92	43·40	1·220	3020	63·1	39·4	17·3	27·4
5160..........	IV.......	1700	14·10	45·50	40·40	1 157	1990	60·2	38 6	17·0	24·3
5153..........	V.......	2288	12·68	37·52	4·980	1·271	3425	65·8	39·6	18·0	32·8
5149..........	VI.......	572	14·00	38·70	47·30	1·211	2990	64·7	38 4	17·4	30·6
					HUMERUS.						
5154..........	I.......	1617	9·20	39·20	51·60	1·274	3080	66·6	38·5	17·9	34·4
5157..........	II.......	1310	13·7	45·8	40·5	1·091	2500	61·0	40·7	17·9	24·7
5158..........	III.......	1239	14·40	43·60	42·00	1·181	2800	63·3	39·4	17·7	26·7
5160..........	IV.......	1343	15·10	45·50	39·4	1·148	2480	60·0	40·0	17·7	23·6
5153..........	V.......	1732	11·50	40·60	47·90	1 211	2700	66·0	39·2	17·8	32·6
5149..........	VI.......	1332	12 36	39·90	47 74	1·211	1950	65·0	39·2	17·7	31·1
					METACARPUS.						
5154..........	I.......	449	11·57	21·80	66·63	1·522	2280	66·6	38·5	17·7	44·4
5157..........	II.......	356	12 37	30·92	56·71	1 368	1800	65·8	38·4	17·7	37·6
5158..........	III.......	345	13·80	23·30	62 90	1·395	1200	65·5	38·7	17·6	41·2
5160..........	IV.......	352	13·50	32·2	54·3	1 375	1375	62·7	39·3	17·9	34·1
5153..........	V.......	443	11·75	23·75	64 50	1 477	2650	67 1	38·9	17·8	43·3
5149..........	VI.......	348	13 55	21·62	64 83	1 402	1725	66 16	38·6	17·6	43·1
					RIB.						
5154..........	I.......	305	22·9	16 8	60·3	1·525	—	63·6	—	—	38·5
5157..........	II.......	220	25·5	27·4	47·1	1 257	—	57·9	—	—	27·2
5158..........	III.......	187	28·8	23·2	48 6	1·390	—	59·6	—	—	29·0
5160..........	IV.......	262	31·2	22·5	46·3	1·312	—	54·6	—	—	25·3
5153..........	V.......	358	23·5	19·7	56·8	1·432	—	62·2	—	—	35·3
5149..........	VI.......	249	28·7	21·8	49·5	1·343	—	61·1	—	—	30·2

NOTE.—The bone measurements were the same as those reported in Experiment II.

Table XL.

EXPERIMENT III.

Phosphorus Balance (daily average).

Group I. Bovine 5154.

Week No.	Intake gm.	Outgo gm. Faeces.	Outgo gm. Urine.	Balance gm.	Month of Experiment.	
1	3·93	3·34	·02	0·57	3	Basal Ration.
2	4·02	3·02	·02	0·98	3	,, ,,
3	10·11	1·88	·03	8·20	10	Expt. Ration.
4	10·39	3·21	·05	7·13	11	,, ,,
5	10·39	4·53	·06	5·80	12	,, ,,
6	10·41	4·14	·05	5·22	12	,, ,,
7	10·42	4·02	·04	6·36	14	,, ,,
8	10·30	4·52	·07	5·71	16	,, ,,
9	10·30	4·21	·04	6·05	17	,, ,,
10	10·86	3·87	·08	6·91	18	,, ,,
11	10·92	2·60	·18	8·14	19	,, ,,
12	11·16	3·72	·08	7·36	20	,, ,,
13	11·08	5·25	·10	5·63	21	,, ,,
		Mean for weeks 3–13				
—	10·49	—	—	6·58	—	—

Table XLI.

Calcium Balance (daily average).

Group I. Bovine 5154.

Week No.	Intake gm.	Outgo gm. Faeces.	Outgo gm. Urine.	Balance gm.	Month of Experiment.	
1	4·43	4·05	·62	−0·24	3	Basal Ration.
2	4·52	5·08	·53	−1·09	3	,, ,,
3	22·48	5·52	·20	16·76	10	Expt. Ration.
4	22·54	15·90	·21	6·43	11	,, ,,
5	22·51	13·65	·37	8·49	12	,, ,,
6	22·53	14·20	·38	7·95	13	,, ,,
7	22·69	14·50	·22	7·97	14	,, ,,
8	22·53	9·00	·50	13·03	16	,, ,,
9	22·47	16·00	·41	6·06	17	,, ,,
10	23·72	12·43	·11	11·18	18	,, ,,
11	23·68	13·60	·14	9·94	19	,, ,,
12	23·27	14·20	·06	9·01	20	,, ,,
13	23·54	13·60	·09	9·85	21	,, ,,
		Mean for weeks 3–13				
—	22·91	—	—	9·69	—	—

Table XLII.

EXPERIMENT III.

Phosphorus Balance (daily average).

Group II. Bovine 5157.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	3·93	3·26	·02	0·65	3	Basal Ration.
2	4·02	3·30	·03	0·69	3	,, ,,
3	4·29	2 74	·04	1·51	10	Expt. Ration.
4	2·57	2·21	·06	·30	11	,, ,,
5	3·30	3·45	·06	−0·12	12	,, ,,
6	3·41	4·15	·06	−0·80	13	,, ,,
7	3·42	3·03	·11	·28	14	,, ,,
8	2·52	2·42	·11	− 01	16	,, ,,
9	2·98	3·36	·06	−0·44	17	,, ,,
10	3·71	2·72	·11	·88	18	·53 gm. P supplemented.
11	3 76	2·32	12	1 32	19	,, ,, ,,
12	3 96	3·19	·16	·61	20	,, ,, ,,
13	3·94	3·43	·24	·27	21	Expt. Ration.
		Mean for weeks 3–13				
..	3 44	—	—	·35	—	—

Table XLIII.

Calcium Balance (daily average).

Group II. Bovine 5157.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	4·42	4·53	0·52	- 0·63	3	Basal Ration.
2	4·50	4·86	0 72	1·08	3	,, ,,
3	22·64	6·00	5 20	11 44	10	Expt. Ration.
4	14·85	5·28	4 70	4 87	11	,, ,,
5	22·90	13·40	2·40	7·10	12	,, ,,
6	22·91	16·25	5·16	1·50	13	,, ,,
7	23·74	17·20	4·86	1·68	14	,, ,,
8	23 03	17·20	3·50	2·33	16	,, ,,
9	22·50	17·40	1·35	3·75	17	,, ,,
10	24·18	11·65	9·00	3 53	18	·53 gm. P supplemented.
11	24·10	13·50	6·08	4·52	19	,, ,, ,,
12	23·81	14·15	4 41	5·25	20	,, ,, ,,
13	24·08	15·10	6·76	2·22	21	Expt. Ration.
		Mean for weeks 3–13				
—	22·61	—	—	4·38	—	—

TABLE XLIV.

EXPERIMENT III.

Phosphorus Balance (daily average).

Group III. Bovine 5158.

Week No.	Intake gm.	Outgo gm. Faeces.	Outgo gm. Urine.	Balance gm.	Month of Experiment.	
1	3·93	3·62	02	0·29	3	Basal Ration.
2	4·02	3·14	03	0 85	3	,, ,,
3	3·11	1·66	·02	1·43	10	Expt. Ration.
4	3 39	2·45	04	0 90	11	,, ,,
5	2·57	2·05	04	0·48	12	,, ,,
6	2·58	2·07	05	0 46	13	,, ,,
7	2·58	2·00	08	0 50	14	,, ,,
8	2 52	3·15	·03	−0 66	16	,, ,,
9	2·52	2 12	·05	0·35	17	,, ,,
10	3·35	2·33	·04	0 98	18	1 07 gm. P. supplemented.
11	3 75	2·28	04	1·43	19	,, ,, ,,
12	3 89	2 44	·06	1·39	20	,, ,, ,,
13	3·02	2 72	·08	0 22	21	Expt. Ration.
		Mean for weeks 3 13				
—	3·03	—	—	0 68	—	—

TABLE XLV.

Calcium Balance (daily average).

Group III. Bovine 5158.

Week No.	Intake gm.	Outgo gm. Faeces.	Outgo gm. Urine.	Balance gm.	Month of Experiment.	
1	4·66	4 87	·46	−0·87	3	Basal Ration.
2	4·54	4·30	·54	−0·30	3	,, ,,
3	44·12	27·45	2·56	14·11	10	Expt. Ration.
4	44·20	26·40	3 24	14·56	11	,, ,,
5	44·03	27·70	4·18	12·15	12	,, ,,
6	44·00	26·70	4·83	12·47	13	,, ,,
7	44·52	27·20	6·32	11·00	14	,, ,,
8	43·80	29·50	1·12	13·18	16	,, ,,
9	43·24	33·55	1·65	8·04	17	,, ,,
10	40·82	30·30	5·59	4·93	18	1·07 gm. P. supplemented.
11	46·14	33·25	4·41	8·48	19	,, ,, ,,
12	46·09	31·90	3·72	10·47	20	,, ,, ,,
13	35·36	24·42	3·21	7·13	21	Expt. Ration.
		Mean for weeks 3–13				
—	43·30	—	—	10·58	—	—

Table XLVI.

EXPERIMENT III.

Phosphorus Balance (daily average).

Group IV. Bovine 5160.

Week No.	Intake gm.	Outgo gm. Faeces.	Outgo gm. Urine.	Balance gm.	Month of Experiment.	
1	3·93	3·21	·02	0·70	3	Basal Ration.
2	4·02	3·58	·02	0·42	3	,, ,,
3	3 11	1·66	·03	1·42	10	Expt. Ration.
4	3·39	2 89	·04	·46	11	,, ,,
5	3 39	1·96	·06	1·37	12	,, ,,
6	2·58	1·91	·06	·61	13	,, ,,
7	3·42	3 01	·04	·37	14	,, ,,
8	3·30	2 98	06	·26	15	,, ,,
9	3 30	2·75	·10	·45	16	,, ,,
10	3·72	2·49	07	1·16	17	,, ,,
11	3 78	2·56	·08	1·14	18	,, ,,
12	3·92	2·89	·19	84	19	,, ,,
13	3·94	3·52	·17	·25	20	,, ,,
		Mean for weeks 3–13				
—	3·44	—		0·76	—	—

Table XLVII.

Calcium Balance (daily average).

Group IV. Bovine 5160.

Week No.	Intake gm.	Outgo gm. Faeces.	Outgo gm. Urine.	Balance gm.	Month of Experiment.	
1	4·47	5·18	·63	−1 34	3	Basal Ration.
2	4 54	4·34	·72	−0·52	3	,, ,,
3	4·18	2·87	·41	0 90	10	Expt. Ration.
4	4·18	3·75	·82	0·39	11	,, ,,
5	4 14	2·56	1 30	0·28	12	,, ,,
6	4·02	3·56	1·29	−0·83	13	,, ,,
7	4 31	3 35	1·26	− 0·30	14	,, ,,
8	3·97	3·25	1·49	−0 77	15	,, ,,
9	4·40	3·25	2·10	· 0·95	16	,, ,,
10	4·64	2·93	1·92	−0·21	17	,, ,,
11	4·57	3·05	1·35	0·17	18	,, ,,
12	3·91	3·24	·43	·24	19	,, ,,
13	4·20	3·61	·27	·32	20	,, ,,
		Mean for weeks 3–13				
—	4·23	—	—	−0·14	—	—

Table XLVIII.

EXPERIMENT III.

Phosphorus Balance (daily average).

Group V. Bovine 5153.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	3·93	2·98	·02	0·93	3	Basal Ration.
2	4·02	3·60	·03	0·39	3	,, ,,
3	11·29	3·05	·04	8·20	10	Expt. Ration.
4	10·39	4·18	·52	5·69	11	,, ,,
5	10·39	2·62	·26	7·51	12	,, ,,
6	10·41	3·82	·16	6·43	13	,, ,,
7	10·42	3·52	·14	6·76	14	,, ,,
8	10·30	5·75	2·05	2·50	16	,, ,,
9	10·30	3·46	2·78	4·06	17	,, ,,
10	10·86	4·52	2·54	3·80	18	,, ,,
11	10·92	3·83	2·18	4·91	19	,, ,,
12	11·16	4·04	1·41	5·71	20	,, ,,
13	11·08	5·85	2·44	2·79	21	,, ,,
		Mean for weeks 3–13				
—	10·68	—	—	5·31	—	—

Table XLIX.

Calcium Balance (daily average).

Group V. Bovine 5153.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	4·40	4·53	·55	−0·68	3	Basal Ration.
2	4·50	4·30	·61	−0·41	3	,, ,,
3	44·17	22·50	·65	21·02	10	Expt. Ration.
4	44·09	26·30	·20	17·59	11	,, ,,
5	44·18	17·50	·19	26·49	12	,, ,,
6	44·17	27·00	·14	17·03	13	,, ,,
7	44·33	30·30	·65	13·38	14	,, ,,
8	44·37	35·30	·88	8·19	16	,, ,,
9	44·25	33·20	·40	1·65	17	,, ,,
10	46·53	36·16	·48	9·89	18	,, ,,
11	46·46	29·10	·41	16·95	19	,, ,,
12	46·15	35·90	·89	9·36	20	,, ,,
13	46·30	32·60	1·87	11·83	21	,, ,,
		Mean for weeks 3–13				
—	45·00	—	—	14·76	—	—

TABLE L.

EXPERIMENT III.

Phosphorus Balance (daily average).

Group VI. Bovine 5149.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	3 93	2·74	03	1·16	3	Basal Ration.
2	4·02	3·54	·02	0·46	3	,, ,,
3	11·29	3·48	1 09	6·72	10	Expt. Ration.
4	10·39	4·14	2·80	3·45	11	,, ,,
5	10·39	3·32	1·70	5·37	12	,, ,,
6	10·41	3·94	96	6·51	13	,, ,,
7	10·42	4·37	2·01	4·04	14	,, ,,
8	10·30	4·83	1·47	4 00	16	,, ,,
9	10·30	4·39	4 08	1 83	17	,, ,,
10	10·86	4 35	4 50	2 01	18	,, ,,
11	10·92	3 37	2 06	5·49	19	,, ,,
12	11·16	3 33	2 30	5·53	20	,, ,,
13	11·08	4·21	4·53	2·34	21	,, ,,
		Mean for weeks 3–13				
—	10·68	—	—	4 29	—	—

TABLE LI.

Calcium Balance (daily average).

Group VI. Bovine 5149.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	4·43	4·27	·72	−0·56	3	Basal Ration.
2	4·51	4·89	·64	−1·02	3	,, ,,
3	4·21	2·85	·17	1·19	10	Expt. Ration.
4	4·11	2·06	·27	1·78	11	,, ,,
5	4·14	2·56	·12	1·46	12	,, ,,
6	4·15	2·82	·19	1·14	13	,, ,,
7	4·39	4·60	·53	−0·74	14	,, ,,
8	4·07	3·00	·26	1·81	16	,, ,,
9	3·93	3·34	·25	0·24	17	,, ,,
10	4·35	2·48	·12	1·75	18	,, ,,
11	4·34	2·73	·16	1·45	19	,, ,,
12	3·87	2·16	·24	1·47	20	,, ,,
13	3·96	2·76	·43	0·77	21	,, ,
		Mean for weeks 3–13				
—	4·14	—	—	1·12	—	—

Section IV.

Plant Physiology

AND

Poisonous Plants.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 10, *Number* 2, *April*, 1938.

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Some Physiological Aspects of the Genus Tribulus.

By M. HENRICI, PH.D., D.SC., Senior Research Officer, Veld Reserve, Fauresmith, Division of Plant Industry.

PREFACE.

IN previous communications by Rimington, Quin and Roets (*Onderstepoort Jnl. Vet. Sc. & Animal Indust.* Vol. 3, No.1, pp. 137-157—1934, and Vol. 4, No. 2, pp. 463-478—1935) it was shown that in the pathogenesis of Geeldikkop as caused by species of Tribulus and other similarly acting plants, the elaboration of an icterogenic principle within such plants should be considered as the primary cause of the condition. When ingested this factor then provoked stasis on parts of the digestive system as well as a severe obstruction in the bile excretory mechanism of the liver which in turn led to the characteristic intense jaundice of the whole body.

The other characteristic feature of the disease, viz., the acute sensitivity to sunlight as shown by marked swelling and later necrosis of all unpigmented and unprotected skin, was shown to be due to the plant porphyrin phylloerythrin which originated as a normal disintegration product of ingested chlorophyll mainly in the forestomachs of ruminants. Traces of this highly fluorescent pigment are normally absorbed from the small intestine only to be re-excreted in the bile thus keeping it strictly within entero-hepatic circulation and to pass out ultimately in the faeces. When, however, obstruction to the bile flow occurs, this pigment with its strongly photodynamic action also finds its way into the systemic circulation and thus also into the cutaneous vessels where in the presence of direct sunlight it is responsible for the acute oedematous swelling usually noted on the facial skin and ears of merino sheep.

Seeing, therefore, that Tribulus plants are on the whole rich in chlorophyll, carotinoids and other related pigments and seeing also that some of these pigments and their breakdown products enter into the aetiology of the symptom complex of geeldikkop, a systematic study was undertaken on the pigment metabolism of Tribulus collected under various climatic conditions. This article is the outcome of this study, treating as it does only with this one aspect of the problem. The other aspect, viz., the elucidation of the primary icterogenic factor is being investigated along other lines and consequently will be reported on separately.

INTRODUCTION.

FOR the farmer in the Karroo and the karroid areas the genus Tribulus is of great interest, as it includes plants which are at times excellent fodder plants and at other times suddenly prove fatal to sheep causing the dreaded "dikkop". It is up to now not all clear whether the causing of "dikkop" is confined only to certain species or, what is more likely, whether all the species may under particular edaphic and meteorological conditions produce dikkop. It is unfortunate that the systematics of the genus is not properly worked out, thus it is often impossible to say which particular species is actually found in the veld and whether other plants which have decidedly another habit of growth are different species or only a physiological variety. According to the literature (Theiler 1918, Quin and Rimington 1933, 1934, 1935) the view is more wide spread that it is a question of physiological state, and not of different systematic species. The author having observed the growth of Tribulus on different veld (alluvial soil, farm yards, limestone, dolerite brown soil and sandveld) was at first rather inclined to think of different systematic species, with their respective hybrids, especially *Tribulus terrestris* and *T. parvispinus*. Since Schweikerdt (1937) most recently puts these two species together again, and only accepts *terrestris*, the view had to be abandoned and in the present paper the different varieties will be called physiological strains. Cultivation experiments which are in progress on the Veld Reserve at Fauresmith ought to bring about a final conclusion.

When Dr. Quin and Dr. Rimington asked the author to collaborate in the Tribulus question not much was known about the actual poisonous principle and every possible poison source in the plant had to be considered. As the Fauresmith district is about on the northern boundary of the area of dikkop outbreaks, outbreaks did not occur regularly every year, and poisonous material could only be collected sporadically and tested for different possible constituents which might have been the cause of its toxicity. In the course of the years more definite views were expressed as to the possible poisonous qualities (Rimington and Quin 1934, 1935). It is clear that with the progress of the work of the Onderstepoort workers the questions put to the botanist changed as well. Thus the results put forward in the Tribulus question are really the product of different views held by these workers. Right from the start it was mentioned (Rimington 1933, 1934) that phylloerythrin played a rôle in dikkop. This led to the investigation of the green plant pigments. The carotinoids were as well investigated, as it was seen that material collected in the veld during the occurrence of dikkop contained an unknown yellow pigment. It was thought that it was not impossible that the breakdown products of the normal carotinoids were in some way connected with the poisonous principle of the Tribulus (Polyterpenes-diterpenes). The question of physiological variety was tackled in culture experiments. An investigation of the anatomy of the plant was also done, as well as some microchemical reactions. All these questions will be dealt with in separate paragraphs.

The Anatomy of Tribulus.

The leaves of Tribulus do not offer any definite peculiarity. In transverse section the following is seen: the epidermis is slightly cuticularized and has stomata on both sides. The stomata on the upper side are slightly elevated. The leaves are covered with trichomes inserted into a pedestal. Below the upper epidermis is a tight layer of long thin palisade cells (1 : 10) which reach to about the middle of the leaf, in the middle are found very large sheaths for the bundle and large cells with calcium oxalate. The cells of the sheaths are exceptionally large. Cutting transversely, bundles may be cut transversely or parallel to the vessels; in both cases the sheath of the bundle is prominent. Below the bundle another layer of palisade cells is found, slightly shorter than those of the top layer, only occasional cells of spongy parenchyma are seen below the lower epidermis.

There are in the middle of the leaf a good few cells of calcium oxalate, but to the author's mind not enough to correspond to the large amount of $(COO)_2$ found in the macroanalysis (see page 384). There must be still some more acid potassium or sodium oxalate in the cells.

Considering the recently advanced hypothesis of the presence of resinic acid in the poisonous Tribulus, it is worth while mentioning the entire absence of any chiliferous vessels containing resin or lysigenous or schizogenous ducts in the leaves, stems and roots If any resinous product is found it must be in the plasma of ordinary cells. On the material collected in 1937 no glandular hairs could be detected, but only trichomes. The leaves did not contain any idioplastes with oily or fatty contents, only the upper epidermis containing small amounts of anthocyan, tinging it purple. Neither the stems nor the roots of Tribulus show any ducts or idioplastes, or cells with oxalic acid. The cortex tissue of the roots has a tendency to work loose from the xylem in a similar way to that described for grasses (Henrici 1929), only no actually dissolved tissue could be found.

Systematics of Tribulus.

After the species of Tribulus found around Fauresmith having for some years been identified as four to five different species (*murex*, *terrestris*, *parvispinus*, *zeyheri* or *cystoides*) today Schweikerdt accepts only the two species *terrestris* and *zeyheri* for the Fauresmith district. Since that time *Tribulus pterophorus* has been found in the district on the banks of the Orange River at Zoutpansdrift. The species *terrestris* is characterised (Schweikerdt, p. 161) by free intrastaminal glands, which are never connate to form a shallow cup; hemispherical stigma which are never slender; petals 2-12 mm. long and sepals 2-6 mm. long. All the other species have intrastaminal glands connate to form a shallow cup at the base of the ovary; stigma slender, usually pyramidal. *Terrestris* seems to be a most plastic species: striking details seen in its growth by the author will be given under Morphology, but for the complete description Schweickerdt's paper (p. 172) should be consulted.

Morphology of Tribulus.

The morphology of Tribulus will be touched only in so far as it seems necessary to elucidate the physiological growth varieties as encountered in the veld. The most striking feature of the three species under observation is no doubt the very small amount of root material in comparison with the corresponding aerial parts. In material collected on the farm Poortjie in the Fauresmith district 30 lbs. of fresh *Tribulus parvispinus* yielded only 1¼ lbs. of roots, for *Tribulus terrestris* on the Reserve itself the ratio was still worse. The plant for morphological reasons is bound to wilt as soon as the rain stops for a few days. And yet Tribulus has two typical forms which differ so much in growth habit that one is inclined to look on them as different species. The one form, so to say, which is encountered always in the Railway enclosure near the Reserve and in rainy weather on the Reserve itself and in the veld is a spreading, many-branched prostrate form. The branches may be 2 metres long, and are usually branched again. The flowers are of medium size, the sepals generally shorter than the petals. This type generally has two different kinds of fruits, typical "dubbeltjies" breaking up into five cocci lengthwise, the cocci having each 4-6 spines, though sometimes only two, and in the middle a crest of bristles and tubercles. Very often not all the five cocci are properly developed, the fourth and fifth having no spines whatever, or the fifth being suppressed entirely, so that the ripe fruit actually consists of only three cocci with spines, and a small fourth without spines. As a matter of fact the author saw more plants with three or four cocci than with five. The whole plant is generally sparingly hairy, the hairs are coarse, sharp and have partly a pedestal. If this plant wilts the secondary branches turn up vertically, as well as the pinnate leaves, which fold up. Another very small type of fruit is at times found on these plants, consisting of 3-4 cocci, practically covered all over with sharp hairs but not with proper spines.

The other type of Tribulus found everywhere in the veld of the Fauresmith area, on limestone or on alluvial or dolerite soil, is a very small plant, generally growing erect or with branches only about six inches long. On limestone especially the plant is absolutely covered with silky hairs. The flowers are generally small, the sepals being as long as the corolla. Very occasionally this type is also found with a medium-sized flower. Leaves and the few secondary branches always stand vertical as soon as it is dry. The completely upright form has never been seen with spread leaves. When dikkop has been reported in the surroundings of Fauresmith, or when plants said to cause dikkop have been sent from the Cape to Fauresmith, this type of plant has always been found. The type, however, is not necessarily poisonous, in drought years it grows in the plots of the Veld Reserve. The cocci of the fruit have sometimes only two spines. Fruit on the whole are not found regularly on this type.

It is quite obvious that in South West Africa or in the other natural habitats of the Tribulus with large flowers these species may prove fatal. But in the south-western Free State they do not occur to such an extent as to be an economic problem, except perhaps *T. pterophorus* on the banks of the Orange River.

Seeds are collected from all possible sources and sown on the Reserve. In the first generation only plants with long stems were obtained, but nearly all had the two kinds of fruit, the large fruit and the small one. As mentioned before on the Reserve generally the two forms are found. They are never poisonous. In 1932 the upright form grew in the worst drought. An uninitiated person could not discover the plants on the soil, but three sheep lived on this plot for six weeks and gained on the average 14 lbs. liveweight in this period.

Just as geel dikkop itself is only confined to a definite area of South Africa (between Victoria West and the north of Fauresmith), although the genus is widely spread, in the area itself the poisoning is only observed on a quite definite habitat. In the Fauresmith district there are only about five farms which are notorious for their regular heavy outbreaks of dikkop. On other farms there may be occasional cases, but never heavy outbreaks. Time and again it has been pointed out that geel dikkop can occur on such different soils, under different conditions. In the Fauresmith district the habitat of poisonous Tribulus seems to be restricted to two soils, limestone formations and sandy (often alluvial) red soil with low water holding capacity, often encountered on dry river banks. The low water holding capacity seems at first sight the only common feature of the two soils. For years on the limestone formation at Poortje, only small forms with short stems and small flowers or the upright form was found. Only in the very wet February 1937 the spreading form with long procumbent branches was recorded. The form on limestone is covered with silvery hairs. The stems are very red.

In going through the masses of Tribulus collected during the last five years, and trying to have it identified, one cannot help thinking it to be a thankless job. Working in the veld and observing the growing habits, the impression is surely obtained that we are not working with well defined species, but with hybrids. The eternal changing of the size of the petals compared with the calyx and the variable fruit and the peculiar two fruit types give enough food for thought in this direction.

Content of Chlorophyll and Derivatives of Chlorophyll in Tribulus.

1. *Method.*

At the same time when Rimington and Quin (1934) found that phylloerythrin was the cause of photosensitisation in sheep it was obviously the thing to look for this pigment in Tribulus. An investigation of the leaf pigments, green and yellow, of Tribulus was undertaken. It was also thought that in case the systematics of the genus should prove inadequate, it would be possible to isolate physiological varieties. In working on the chlorophyll of Tribulus collected in different localities, using the classical method of Willstaetter and Stoll for fresh plants (1913, p. 138 ff.) it soon appeared that large differences existed between plants from different localities, but that a great similarity occurred in the amount of chlorophyll in plants which had similar growth habits and grew on similar soil.

An annual plant such as Tribulus which finishes its growth and reproduction within a few weeks to three months, can be expected to have large changes in its chlorophyll content during the season. To study these changes regular samples of two Tribulus varieties were taken, the one of a very luxuriant strain growing in the railway enclosure below the Reserve, which always had long procumbent stems and belonged to *T. terrestris*. The other strain was collected on the Reserve itself, it was also a procumbent plant, but never so juicy as the first strain. Then in and out of the Fauresmith districts where outbreaks occurred within the reach of an officer connected with the Tribulus investigations, as many samples as possible were collected and analysed, careful notes being taken of the growth habit, water content and if possible of fruit and flower of the particular strain. A few samples from Victoria West had to be analysed in the dry state, as they arrived after a long journey. The standard used for the work was obtained by the courtesy of Professor A. Stoll, Basle, being a preparation of copper chlorophyll, corresponding in intensity to 120 per cent. of the same amount of chlorophyll. 0·038 grams of copper chlorophyll were dissolved in ether, saponified with methylalcoholic potassium, washed down with water, and the final solution made up to 1,000 c.c. Then comparison was made in a Leitz colorimeter.

The sampling of the plants was done as quickly as possible into closed jars, and a sample in a closed weighing-bottle set aside already in the veld for the determination of fresh matter. The leaves were ground immediately with a little chalk and pumice stone and extracted in the usual way (Willstaetter and Stoll 1913, p. 138 ff.) When there was much chlorophyll present, as in the series of November 1934 after heavy rains, the pigment came down in 35 percent. acetone and had to be regained with ether, small amount of the 35 per cent. acetone being shaken with a fair volume of ether. In all other cases the separation went very smoothly, only the very wilted plants offering some difficulty in the final separation.

Besides taking quantitave readings of the amount of chlorophyll, the solutions were tested with a Zeiss hand grating spectroscope, the bands being compared with the existing ones in the literature. Most solutions made up of 10 grams fresh leaves were far too dark for direct observation and had to be diluted 12-30 times.

For the purpose of ascertaining the position of the bands, separation of chlorophyll a and b was necessary. At the start this was done by Willstaetter and Stoll's method (1913, p. 153 ff.) Later is proved quicker to separate with an activated sugar column (Winterstein 1932, p. 1402, and Treibs 1932, p. 1351). As chromatograms were used for the separation of the carotinoids, most of the later separation work on the pigments was done in this manner. The icing sugar was heated to 150°C to activate. It proved advisable to cover it with a thin layer of talcum. The total column was much longer (30 cm.) and wider than the columns for the yellow pigments. Chlorophyll a was retained partly in the talcum, partly by the top layer of sugar in three layers. Chlorophyll b went through. As solvent equal parts of benzene and petrolether were

used. The chromatogram was eluted with ether plus 1 drop of ethylalcohol. All decomposition products if present went through with chlorophyll b.

Content of Chlorophyll.

From the start it was obvious that the fresh green plants from the railway enclosure with a high water content—nearly unknown in South African plants—had absolutely normal chlorophyll in large amounts (Table 1). But within a few weeks the content of water and chlorophyll calculated on dry matter decreased and adjusted themselves to South African conditions, the plant became more solid, the chlorophyll content became similar to that of other areas, although being always a so called strain with higher chlorophyll content. The strain from the reserve had much less chlorophyll, it showed however the same seasonal variation, higher in the young plant, decreasing slowly, even keeping stationary under the influence of good rains, and decreasing rapidly at the end of the season. An intermittent drought always resulted in a decreased chlorophyll content, behaviour which is already known for South African plants (Henrici 1927). Contrary to the series of the railway enclosure, the plants wilted badly and in no case the water content was high.

The plants collected on the different farms in the district Fauresmith, at Onbekend (Middelburg district, Cape), or at Victoria West belonged to strains either with low chlorophyll content or with a content of 1-1·5 per cent. They all showed the seasonal decrease.

Although most of the Tribulus strains collected during outbreaks of dikkop had a small chlorophyll content of 0·08-0·5 per cent. of the dry matter, not all the strains with a low chlorophyll content actually occur on veld where dikkop is observed. Furthermore it is interesting to note that all strains with a medium and high chlorophyll content were not collected where a dikkop outbreak occurred. A priori one would think that when a cleavage product of the chlorophyll is one of the responsible causes of dikkop, individuals which contain a lot of chlorophyll should cause dikkop. The present investigation shows clearly that this is not the case. The collection and working up of Tribulus during an outbreak of dikkop was always done so quickly that no material losses of chlorophyll could occur. In the Fauresmith area Tribulus dikkop is only produced by wilted Tribulus, but not all wilted Tribulus produced dikkop. In more Southern areas dikkop may be apparently produced by plants showing only incipient drying. Wilting certainly decreases the chlorophyll content, but even in the fresh state the chlorophyll content of a strain which later proves fatal in the wilted state, is never high. Table 1 is very instructive on this point.

It must be emphasized that the wording high, medium and low chlorophyll content is relative and only applies to Tribulus. The figures often encountered were 1·5, 0·5 and 0·08 per cent. chlorophyll a+b per dry matter. Willstaetter (1924, p. 15) gives for Sambucus chlorophyll a+b 0·93 per cent. on dry matter basis, which is a fair mean between the two higher values encountered. The value 0·08

is even in an absolute sense very small, the enormous value of over 5 per cent. is certainly quite exceptional and can only be explained by the chlorophyll formation taking place much quicker that the formation of any solid substance. All other values are absolutely normal for the particular strain.

So far only the actual amount of chlorophyll has been considered. With regard to purity of the chlorophyll, it was obvious from the start that the wilted plants which caused dikkop contained some other pigments besides the chlorophyll. The acetone extraction was not bright green, but had a decided olive, even brownish tint. The chlorophyllines were not pure although the major portion was still normal for the red bands and on saponification the normal brown phase was obtained. In the green blue part of the spectrum a sharp and strong band could be detected in place of the ordinary weak band of chlorophyll. The band extended from about 5250-5400 Angström, varying somewhat according to concentration. This band was never observed in a non-poisonous Tribulus with low chlorophyll content. The non poisonous Tribulus always showed the normal chlorophyll spectrum, Willstaetter's band 6 rather weak, if visible at all. The author would be inclined to look for the pigment causing this band in the phaeophorbides or phaeophytin, for which a strong band in this position is characteristic, but the second strong band for phaeophorbides could not be traced. For the time being the only conclusion which could be drawn was that there were cleavage products present in wilted Tribulus, which causes dikkop, but only in those. An observation may be mentioned here. If after some months of storing the plant powder of Tribulus was used again to isolate chlorophyll, the pigment of the fresh or wilted non poisonous Tribulus was normal, in percentage perhaps a little less after years of storing. Never was any sign of deterioration seen, whilst when the Tribulus which had caused dikkop was treated in the same way, very little, often only traces of chlorophyll were left after a few months' storing, and a fair amount of decomposition products were present. As the plants were treated in absolutely the same manner, it can only be suggested that something in the plants collected during outbreaks of dikkop caused the difference. The question of Tribulosis is so complicated that smallest indications of that sort have to be taken notice of.

Another point was clear as well. Phylloerythrin was not present in the wilted nor in any of the Tribulus plants. To test this, the plant material (fresh, green, wilted, wilted poisonous Tribulus) was extracted with a mixture of ether and acetic acid (2 : 1), and the extract shaken with acid of different strengths. The acid extraction did not show the spectrum of phylloerythrin.

The presence of phaeophytin was considered, as this pigment is supposed to occur in dry plants, for instance in herbarium specimens. Lately it has been recorded in leaves under the influence of excessive heat or cold (Röben 1933, Röben und Stern 1935).

The colour of phaeophytin made by treating the chlorophyll with concentrated acid agrees with the colour of the pigment in the wilted poisonous plant.

It is therefore quite likely that phaeophytin is present in the poisonous wilted plants. Phaeophytin is derived from chlorophyll by the action of acid, especially easily by oxalic acid which splits off the magnesium of the chlorophyll molecule. Would such a reaction be possible in the living plant? Mineral acids are of course out of question, but oxalic acid is present in Tribulus, and what is rather interesting, the amounts present in older wilted Tribulus are about four times as high as those in fresh Tribulus. The Tribulus from the Railway enclosure contained in the first determination 0·21 $(COO)_2$, while wilted Tribulus contained so far 0·9-1·3 per cent. The oxalic acid was determined by Dakin's modifications of Salkowski-Autenrieth and Barrth's method (Hawk 1927, p. 769 ff.), the Calcium by the usual method of MacCrudden.

Cross sections through the leaves were treated with H_2SO_4. In the few crystal druses to be seen—there were remarkably few—gypsum needles were observed. Part of the oxalate is certainly present as calcium oxalate, but is does not seem all. Tribulus leaves contain a lot of calcium, between 2·5-4·5 per cent. CaO; it would be more than enough to neutralize the oxalate, but apparently most of it is not bound to $(COO)_2$ or more crystals should be encountered, and the plant juice should be less acid. It is likely that acid K or Na salts of oxalate are in solution which during wilting penetrate to the chlorophyll and destroy it. There is no doubt that during wilting the semipermeability of the protoplasm is changed to permeability, and the acid salt obtains access to the pigment in the chloroplasts, and split the magnesium off. So much for the plant. It has been maintained that such a decay of the chlorophyll would immediately be stopped in the stomach of the sheep, as the first enzyme pepsin only acts in acid medium. This may be so, but by that time the cleavage product is already irreversibly formed, and the flora of the intestinal tract need only continue their work to prepare phylloerythrin from phaeophytin (Rimington and Quin 1935).

To separate phaeophytin quantitatively from the chlorophyll, will be the next step to be taken. Phaeophytin however is not the only cleavage product occurring in Tribulus during wilting. To get an idea what the breakdown products in the plant were, the method of Willstaetter and Stoll (1913, p. 262-273) was adapted, the ether solution of the plant pigments being extracted with hydrochloric acid of different strengths. (Tables 2 and 3.)

It was fully realised that under the influence of concentrated acid phaeophytin may be formed; but it was obvious from Willstaetter's table that this would only happen with acid of 29 per cent. or over, that is to say, no interference was to be feared with lower concentrations. The point was tested with crystallized chlorophyll dissolved in ether. With no strength of hydrochloric acid did a change of colour take place in the ether. The acid up to 28 per cent. did not extract any pigments from the ether. 29 per cent. HCl was very slightly coloured green. The result is exceedingly interesting (Table 2). No Tribulus from the railway enclosure nor from the reserve (although some of the latter was wilted), tinted weak 8 per cent. acid; but half of the wilted Tribulus which was

collected during outbreaks of dikkop tinted it faint blue green. 16 per cent. HCl was tinted blue green by all but one of the poisonous wilted plants and tinted green (a different shade) by two pigments of wilted not poisonous plants. 22 per cent. HCl took a blue green pigment from all wilted plants. Some extracts from fresh plants gave a colour to 22 per cent. HCl, but the shade was a different green, the same colour as was obtained with concentrated acid from all chlorophyll and which is due to phaeophytin. With 22 per cent. HCl in several cases the ether could be completely extracted, being absolutely yellow from the carotinoids.

Summarizing it can be said that there are certainly cleavage products of the chlorophyll present in the wilted Tribulus collected during the outbreaks of dikkop and the plant possesses in this stage a constituent which breaks down the entire chlorophyll. In the isolation of chlorophyll, they cling to the pigment to the very last stage. Run through the sugar column they cling to chlorophyll b. Another means has still to be found to separate them.

Content of Carotinoids.

1. *Method.*

Although is was soon obvious that the icterus of the dikkop was due to bilirubin (Quin 1933, p. 505 ff.) and not to a plant pigment, as first suggested, it was thought of interest to know what yellow pigments were present in Tribulus, the more so as the carotinoids are also built up by the isopren radical, and constituents of isopren (resinic acid) may play an important rôle in the poison principle of Tribulus (Rimington and Quin 1935 b). At the time when it was certain that no plant pigment causes the icterus, the present investigation was already far advanced and certain results obtained. The pigments were investigated quantitatively and spectroscopically. Either fresh plant material or carefully air dried leaves were used for the isolation of the yellow pigments.

First Willstaetter and Stoll's method (1913, p. 231 ff.) was used.* The bichromate standard was used and compared with the standard of Azobenzol. A Leitz colorimeter was used, but in spite of all precautions, the colours did not quite match and the colour filters of the colorimeter were too dark to allow comparable readings in any other colour than yellow. For no apparent reason the azobenzol readings were too low. In a paper of Guilberts (1934) a standard for carotinoids was described which agreed well with the values of the bichromate standard, and which was in tint of colour exactly the tint of the carotene prepared from plant leaves. It consisted of 3·06 grams of Naphtol yellow S and 0·45 grams of orange G. dissolved in 1 litre of water as stock solution. Of the stock solution 5 c.cs. are diluted to a 1000 for the standard. The ratio tables of the bichromate standard could be used for this standard as well, for the calculation of xanthophyll. As this standard proved to be the best as colour match, in the later work is was solely used.

* As for the chlorophyll determination the method will not be described in full detail, the original being closely followed. Only when alterations proved necessary or when difficulties were experienced, these are fully described.

Different extraction methods were tried out during the investigation, especially when only carotinoids and not the chlorophylls were used. Besides Willstaetter and Stoll's method (1913) Zechmeister's (1932 and 1934) extraction and separation were used. As his tables are at times difficult to follow, the method will be more fully described. The main point with Zechmeister's method is the first extraction which is alternatively done with petrolether boiling point 60-80°C. and methylalcohol. 10 Grams of air dried plant powder were shaken in a wide mouth stoppered bottle alternatively with small quantities of the two solvents and the two solvents being finally united. The amount of alcohol must be measured carefully so that the water content can be made up to 10 per cent. The exhaustive extraction needed generally more solvent (600 c.cs.) than Willstaetter prescribes. It may be mentioned here that in different prescriptions for the extraction of plant material the amount of solvent to be used varies a few thousand per cent. Even one of the Willstaetter prescriptions for a large scale extraction speaks only of six litres solvent to 2 kg. of plant material (1913, p. 76) while in the smaller extraction 300 c.cs. of solvents are used to 10 grams fresh plant material. To have an extraction as complete as possible, the author had always to use a fairly large amount of solvent. The combined petrolether-methylalcoholic extract (extract I) was separated by adding the necessary water to make the methylalcohol 90 per cent. Most of the chlorophyll went into the fraction of petrolether (I), the alcohol fraction (I) was repeatedly shaken with petrolether (II) which sometimes removed all the chlorophyll. All petrolether-extraction as well as all methanol fractions were united respectively. At this stage the latter were often very cloudy but cleared sometimes on standing overnight. Under these conditions the xanthophylls are in the alcohol phase, and the carotenes in the benzine phase. If lypochroms or lycopin should be present, they would be in the benzine phase; it may be already stated here that none was found in the leaves of Tribulus.

The methylalcoholic fraction (I) is then saponified in case there is still some chlorophyll present which can easily be controlled in the spectroscope. In case no or very little chlorophyll is present, only 1-2 c.c. 2N NaOH is added; as plenty of flavones are present, the solution becomes cloudy and reddish yellow. When more chlorophyll was present, up to 20 c.c. NaOH were given. Experience teaches very quickly how much has to be added so that the brown phase appears immediately. The solution was left to itself for about three hours. After three hours the same amount of water as methylalcohol is added to the saponified solution and then the emulsion shaken with benzine. If chlorophyll is present, it is hypophasic and stays back in the methylalcohol-water fraction II. If no chlorophyll is present, this fraction II is yellow or yellow-red from flavones, some of them flocculating out at the contact surface between alcohol and benzine. It proved advisable to extract the large bulk of the methylalcohol layer II several times with small amounts of benzine, till the latter remained colourless after good shaking. The methanol layer II certainly contained some benzine in the emulsion, a good deal of it cleared and united with the benzine layer II. All these benzine extractions were united to extraction III, which contained all the xanthophyll (in the sense of Willstaetter and Stoll 1913).

The petrolether fraction 1 which contains chlorophyll and carotenes is mixed with an equal volume of 5 per cent. ethylalcoholic potash and kept for three hours at 40°C. An amount of water corresponding to 20 per cent. of the alcoholic potash was added to separate the layers. The benzine layer is repeatedly shaken with 90 per cent. methanol, until the latter remains colourless. It happened several times that the benzine fraction I did not mix well with the potash and consequently the saponification was incomplete. More potash was added and chlorophyll again washed out. But after a few tentative trials it was found much easier to remove the remaining unsaponified chlorophyll by shaking the benzine fraction I with some talcum plus some glowed Na_2SO_4.

The talcum immediately absorbed the chlorophyll and pure carotene solutions were obtained. Xanthophyll and carotene were compared with the naphtol yellow S standard described before.

In going through the literature, the method of Guilbert (1934) was encountered and consequently tried out. At first it seemed rather drastic to use boiling alkali in the separation, as every paper on carotenes contains a warning that these pigments are exceedingly sensitive to heat. As on the other hand the method seemed very time saving, it was worth while trying out. After some experimenting the method gave satisfactory results, differing very little from those obtained with Willstaetter and Zechmeister's method. The main point—in all three methods—is the very thorough grinding of the sample.

Willstaetter and Stoll's method (1913) is described so well, that scarcely anything has to be added. The only point which at first offered some difficulties was the final removal of the xanthophyll to the ether where it is stated that water should be added slowly. The author found it more satisfactory to add a very large quantity at once. The xanthophyll separates immediately out into the ether, although some ether may be absorbed in the water. If the water was added slowly, emulsions occurred which had to be taken up several times with ether.

The quantity of the carotinoids was determined in a Leitz colorimeter. As certain colours were observed in the freshly collected plant, it seemed advisable to check the pigments in the spectroscope. In the literature the bands of these pigments are mostly given for solutions of alcohol, benzene, chloroform and carbondisulphide. The chromatogram method (Zechmeister 1934, p. 98 ff) was used to separate the carotinoids.

As absorbent, activated MgO prepared from $Mg(OH)_2$ was used, as no suitable aluminiumoxide could be obtained (Strain H.H. 1933). The different components of the xanthophyll were adsorbed generally in four different layers and the carotene could be far enough removed from the xanthophyll or could be forced to pass the column unadsorbed, at will. The yellow pigments in the ether solution were fanned to dryness, taken up with benzene, or a mixture (1 : 1) of benzene and petrol-ether. Then the solution is run through the column. After the adsorption column had been sucked dry, it could easily be removed from the glass tube with a rubber stopper

fixed on a glass rod. This device was also useful in filling the column, but care had to be taken that the stopper is *less* in diameter than the tube so that in pulling out the stopper, no vacuum occurs which may break the column. The column was usually 10 cm. long, and ± 1 cm. in diameter.

After the column has been taken out of the tube, the different layers are separated (see results) and eluted in the centrifuge. Generally 2-3 stirrings with the eluting fluid and centrifuging were enough. If the pigments are to be used for spectroscopic work, elute with ether and 1 per cent. alcohol. For colorimetric work elute with ether or petrolether. For spectroscopic work the elution is fanned down again and the pigment taken up with the solvent in which the bands have to be measured. This takes only a few minutes. At this stage, from the more concentrated solutions crystals were obtained.

The fanning down was done with an ordinary electric fan to which a long funnel made of stiff brown paper was attached, guiding the air to the surfaces of the solution. The last traces of liquid after fanning could easily be removed by putting the crystallising dish into a desiccator with $CaCl_2$.

The absorption bands were studied in the usual way in the new Zeiss spectroscope, opening of the slit ½ (0·05 mm.), thickness of layer 10 mm., carbondisulfide, benzine and ethylalcohol being used as solvents.

2. *Results.*

I. *Spectroscopical. Table* 4.

As the concentration of the yellow pigments varied a lot in the different plants, the chromatogram did not allow of distinguishing all the bands in all cases. But in the typical MgO column of fresh or wilted not poisonous Tribulus five distinct bands (including carotene) could be seen. In the following the counting is done from the bottom to the top. Sometimes a faint yellow band preceded the actual pink band which proved to be carotene β. This faint band was too weak to be studied with certainty (carotene α?). Band 2 (pink yellow) was very strong and after eluting had to be diluted. It gave the usual absorption band in CS_2 of 521 and 485·5 (centres) and in benzine 483·5 and 452, corresponding to carotene γ β. Carotene was never observed.

The third band of chromatogram, yellow pink, was generally very dilute and showed in petrolether absorption bands with centres at 435 and 458·0 $\mu.\mu.$

The fourth band was the strongest. It was dark orange with a ring of lighter yellow in front of it. Eluted and evaporated down it formed at times good crystals, dark red brown, thickish prisms with swallow-tailed ends, sparingly soluble in CS_2. The absorption bands had the following centres 446; 475·4; 508·2. The band at 420 was not detected. This pigment seems to be Lutein.

Band 5, orange red, was not present in all the preparations. In No. 1046, however, it even formed crystals, like flat (plate-like) prisms. The following are the centres of the band in CS_2: 450·0

(not too good); 478·7; 511·6. It is doubtful whether this pigment corresponds to any known xanthophyll, perhaps it is Zeaxanthin.

The sixth band of the chromatogram, greenish brown-orange, showed very sharp bands in CS_2. The centres were 440; 467·8; and 500·6 (others 440; 470·6: 501·1). The pigment did not crystallize. The bands are those of Violaranthin.

These pigments were obtained from air dry material, a few months old. It seems that in the fresh Tribulus all normal yellow pigments and no unknown pigments are present. When wilted Tribulus collected during outbreaks of dikkop was extracted on the spot the result was different. Unfortunately at that time the technique of the chromatogram was not yet worked out, and only spectroscopic tests on ether solution could be done For the last four years no dikkop outbreak has occurred in the Fauresmith district. The ether solution of the poisonous Tribulus was not yellow but reddish. It was made quite sure (in the spectroscope) that no traces of unsaponified chlorophyll were left which might give to the ether a reddish tint. The usual absorption bands of carotinoids were found and then a very strong band extending nearly to 550 $\mu.\mu.$, from about 520 $\mu.\mu.$ A less dark band was between 500 and 520 $\mu.\mu.$ There is no doubt that there is a pigment in the wilted poisonous Tribulus which is not present in the fresh plant. But this pigment so characteristic in its colour and absorption bands visible even to a lay man in the matter, is not stable. When the air dried plant is extracted a few weeks later, no trace can be found, but, and that is the peculiar point, a pigment is found in the chromatogram which gives the absorption spectrum of Zeaxanthin,

in CS_2 with the centre of absorption 520: 483; 450.

in C_2H_5OH with the centre of absorption 483; 451; 423.

In petrolether long needles were obtained. In methylalcohol long prisms were obtained with swallow tailed ends and sharp edges (see Zechmeister p. 292). If enough poisonous material comes to hand further investigations will have to show whether the disappearance of the unknown pigment and the appearance of Zeaxanthin is a similar case to that described by Heilbron and Phipers (1935). According to these authors fucoxanthin can only be isolated from fresh plants. Ten days after the collection of the Algae no fucoxanthin is found, but zeaxanthin appears, as a product of reduction. Tribulus is a very active plant with a high oxyreduction potential (it needs only the presence of water to reduce nitrates to nitrites in the plant powder): thus the reduction of the unknown carotinoid to zeaxanthin does not seem far fetched, but a very likely occurrence. In normal fresh or slightly wilted Tribulus zeaxanthin was never found. Zeaxanthin is certainly not a usual product in the plant leaves (Zechmeister 1934, p. 184 ff.). It cannot be accepted that the few Tribulus collected during outbreaks of dikkop were especially badly treated (on the contrary, they were cherished); so that a decomposition product occurred. Zeaxanthin is certainly a post-mortem product in this particular case, but would also appear under the best conditions of drying. Here a further observation has to be started, when material (and lots of material) is available.

In this connection Lippmaa's (1925) work on rhodoxanthin has to be mentioned, as this pigment shows a strong absorption band between 515-535 $\mu.\mu.$, while the other three absorption bands (two in the red and yellow part of the spectrum) are much weaker. According to Lippmaa rhodoxanthin occurs in the most different families under definite exterior and interior conditions, such as strong illumination, drought, plenty of sugar instead of starch, etc. All these conditions would be fulfilled in the case of wilted Tribulus, as during wilting starch is hydrolysed to sugar (Iljin 1922), but the absorption band in red and yellow was never observed. This would exclude right away Lippmaa's pigment, if Kuhn and Brockmann do not give different bands for the same pigment. Their bands in ethylalcohol and ether agree much better with the ones seen by the author in the spectroscope. Thus there is a likelihood that the unknown pigment may be rhodoxanthin.

II. *Quantitative Study of the Carotinoids. Table 5.*

In their classical investigation Willstaetter and Stoll (1913, p. 111 ff.) state that under normal conditions in green leaves the ratio of green to yellow pigments shows but small fluctuations, being about 3-6. For very young and Autumn leaves this ratio is different (Willstaetter and Stoll 1915, p. 526) when the amount of yellow pigments is relatively larger.

The question arises as to the ratio of the pigments in the investigated Tribulus. A priori it may be said, that only in a few cases can the plant investigated be regarded as in a normal state; generally they are wilted. If desiccation affect the ratio, differences have to be expected. In Table 5 the total chlorophyll content, the percentage and the ratio of the yellow pigments are given. In a special column the condition of the plant (fresh, wilted, ollected during outbreaks of dikkop) is marked. Table 5 shows beyond doubt that under the given circumstances the ratio of the green to the yellow pigments is not constant, varying as a matter of fact from 8-66.

At first sight it might be thought that the extraction was incomplete, but the analyses were repeated with three different methods (see above) and the values obtained showed very small differences. The greatest care was of course taken to extract as completely as possible. Other plants were tested and gave lower figures (but also higher than Willstaetter and Stoll's), but wilted plants always gave high ratios.

A closer inspection of Table 5 certainly reveals the fact that the content of carotinoids is very low indeed. After what has been said about the destruction of chlorophyll in the wilted plant, it is not surprising that in very badly wilted plants the ratio of green to yellow pigment is relatively low, as apparently chlorophyll and carotinoids have been destroyed. Then there seems to be a group of plants which still have their normal chlorophyll content, but in

which a lot of yellow pigments have been destroyed; they show the widest ratios. There are also a few fresh plants with ratios between 10 and 20, which seem to have a fairly normal content of yellow pigments and chlorophyll.

Summarizing the Table it seems that even in the fresh plant the ratio is wider than in Europe, and that in wilting or even incipient drying which is not yet obvious to the eye, carotinoids (especially xanthophyll) are destroyed before a destruction of chlorophyll takes place, and hence the high ratio; then when the plant is badly wilted chlorophyll and further carotinoids are destroyed and smaller ratios are again obtained. From the percentage figures it seems that especially xanthophyll is destroyed. What actually happens to the pigment, one can only speculate. It is possible that isopren rests are freed and used to build up some other terpenes.

One point has to be stressed. The destruction of the pigments in Tribulus is very unlike the autumnal destruction of plant pigments in Europe, when generally first chlorophyll and its enzyme are destroyed, and then only the yellow pigments. Here just the opposite takes place. The only similarity is that in both cases a lot of water soluble yellow pigments occur (flavones according to positive reaction with caustic alcohol). The difference in both processes may be due to the lack of moisture in the South African climate.

Nitrate and Nitrite Content of Tribulus terrestris.

(Table 6.)

Tribulus terrestris is certainly a nitrate plant. Table 6 gives the accumulated data in this respect. It has to be emphasized that the amounts obtained in any material collected in Fauresmith were much smaller than those obtained in the surroundings of Pretoria by Rimington using the same method (Strowd 1920). As soon as the plants were immersed in water, nitrite appeared, these amounts also being smaller than those of Pretoria plants, the latter probably due to the fact that at Fauresmith the plants were carried into the laboratory and immediately worked up or put into water in the veld, on the whole more in their natural condition with no time lost to allow for chemical changes. The lower nitrate content is due to the poverty of Fauresmith soil in nitrogen. Most Fauresmith pasture plants, except when very young are rather poor in protein.

In spite of the nitrite content of the Tribulus, this radical is certainly not the cause of any poisonous quality of the plant, as our Reserve plants which were never poisonous to stock, always showed positive reaction to Griess' nitrous acid reagent. (Treadwell II 1924, p. 306.)

Content of Calcium and Phosphorus.

In grazing experiments on the Reserve in 1932 *Tribulus terrestris* proved to be an excellent fodder plant, in spite of the poor and wilted appearance; two sheep grazing for two months on a

half-morgen plot with nothing else but Tribulus in it increased 10 and 14 lb. Some determinations of phosphorus were done on Tribulus collected from different places. The phosphorus is very high as the following figures show:—

No.	Date.	Place Where Collected.	Percentage P_2O_5 on Dry Matter.
1221	1/12/34	Railway enclosure near Reserve..............	0·68
1232	28/12/34	Ventersvlei–Fauresmith District..............	0·91
1240	25/ 1/35	Railway enclosure near Reserve..............	0·60

The calcium content of all the samples investigated was very high.

No.	Date.	Place Where Collected.	CaO.	MgO.	
1219	19/11/34	Railway enclosure near Reserve	3·69	1·05	
1221	1/12/34	Railway enclosure near Reserve	3·78	1·08	
1240	25/1/35	Railway enclosure near Reserve	4·45	—	
1220	29/11/34	Reserve........................	3·06	0·90	
1237	22/1/35	Onbekend, Middelburg District.	2·55		Upright, after dikkop outbreak.
1238	22/1/35	Onbekend, Middelburg District.	3·26	—	Prostrate.
1239	22/1/35	Onbekend, Middelburg District.	3·26	—	Prostrate.
1241	28/1/35	Riet River............	4·03	—	Prostrate.
1242	28/1/35	Dassiespoort near River........	4·06	—	Upright.
1245	28/1/35	Dassiespoort near River........	4·52	—	Prostrate.
1243	28/1/35	Dassiespoort Garden..........	3·57	—	Upright.
1244	28/1/35	Dassiespoort Garden..........	3·11	—	Prostrate.
1250	21/1/35	Victoria West "A".........	3·59	—	Wilted, collected during dikkop outbreak.
1251	21/1/35	Victoria West "B"..	3·27	—	" "
1252	21/1/35	Victoria West "C"...........	3·78	—	" "

The magnesium content of the plants, done only on a few samples, was $\frac{1}{3}$–$\frac{1}{4}$ of the calcium content. No relation can be seen between habitat and calcium content. The ratio of calcium content to magnesium is very good. From the point of view of phosphorus it can be understood that *Tribulus terrestris* is excellent food.

Content of Oxalate in Tribulus.

As already pointed out in the anatomical description, *Tribulus terrestris* contains in its leaves a layer of cells with calcium oxalate.

The oxalate was determined macrochemically (Hawk 1927, p. 799), and the calcium oxalate also microchemically (Tunmann 1913, p. 141).

No.	Date.	Habitat.	Percentage $(COO)_2$	
815	31/10/32	Tietiespan	0·78	Upright, wilted, collected during dikkop outbreak.
1042	26/ 1/33	Veld Reserve	0·83	—
1220	29/11/34	Veld Reserve	1·29	Dry.
1219	19/11/34	Railway enclosure Fauresmith	0 63	Spreading.
1221	1/12/34	Railway enclosure Fauresmith	0·21	Very spread.
1235	28/12/34	Railway enclosure Fauresmith	1·14	Spreading.
1240	25/ 1/35	Railway enclosure Fauresmith	1·11	Spreading.
1228	28/12/34	Riet river bank, Dassiespoort	1·25	Spreading, fresh.
1229	28/12/34	Riet river bank, Dassiespoort	0·37	Upright, fresh.
1242	28/ 1/35	Riet river bank, Dassiespoort	1·32	Spreading.
1226	28/12/34	Dassiespoort Yard	1·01	Spreading.
1227	28/12/34	Dassiespoort Yard	0·29	Upright.
1234	28/12/34	Dassiespoort Garden	0·09	Spreading, very much. Fresh as 1221.
1231	28/12/34	Koksfontein, on lime stone	0 47	Upright.
1232	28/12/34	Ventersvlei	0·92	More spreading.
1233	28/12/34	Riet River Bridge	0·83	Little spreading.
1241	28/ 1/35	Riet River Bridge	1·11	Spreading.
1237	22/ 1/35	Onbekend, Middelburg District	0·94	Upright.
1238	22/ 1/35	Onbekend, Middelburg District	1·21	Prostrate.
1239	22/ 1/35	Onbekend, Middelburg District	0 76	Fresh, spreading.

The above table shows the amount of oxalic acid. It varies a good deal. For absolutely fresh plants it is very small (1221; 1234); on the other hand it may be low for upright forms (1231, 1229, 1227). No relation between poisonous qualities of the Tribulus and its oxalate content could be detected. It must, however, not be forgotten that the oxalic acid if not bound to a mineral may at times tackle the chlorophyll when through external conditions the permeability of the cells is altered.

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APPENDIX.

PROTOCOL OF CHLOROPHYLL EX "TRIBULUS TERRESTRIS, NO. 1253, POORTJIE, NOT MUCH WILTED. 25/2/1935.

Slit of spectroscope ½; daylight, depth of layer 12 m.m. The chlorophyll was prepared of 50-gram dry plants, but then diluted about 1,000 times. For certain bands a further dilution was necessary. Chlorophyll (*a*) and (*b*) were separated by a chromatogram of talcum and activated icing sugar.

Chlorophyll (*a*) gave 3 distinct absorption bands. In ether. No. 1 the lowest, No. 3 the highest in the chromatogram. The different absorption bands gave different absorption bands which all corresponded very well with Willstaetter's bands.

Band centres.

No. 1. End absorption 4328 and 7200.
Bands 4100; 4328; 6646; 6600-6702.

No. 2. 5360; 5800; 6168; 4649; 4310; 6600;-6710. More dilute 5100 (faint) 6200.

No. 3. 5350; 5100; 5720 (good); 6130.
6571-6695 more diluted; end absorption 4301 and 6800 faint band 4550.

Chlorophyll (*b*) in benzene.

5350 (band 6, weakest band); 5345; 5643-5861 (band 5).

Further diluted 6154 (band 3); 4782 (band 4); 4665 (band 8); 6628 (band 1).

The bands below 4600 could not be seen well.

The band numbers in brackets are the numbers of Willstaetter.

The observed intensity order is Bd. 6; Bd. 5; Bd. 3; Bd. 4; Bd. 8; Bd. 1.

TABLE I.

Chlorophyll Content (a + b) of Tribulus terrestris from different localities.

No.	Date.	Locality.	Remarks.	Chlorophyll content in percentage of dry matter.	Water content in percentage of fresh matter	Special remarks on the spectre of the chlorophyllides. Figures for normal spectres are not given. All bands taken layer 12 mm.; slit ½, 0·05 mm. daylight.
1032	10/12/32	Veld Reserve	—	0·38	42·3	—
1033	29/12/32	Veld Reserve	—	0·21	40·0	—
1034	9/1/33	Veld Reserve	—	0·59	60·2	—
1042	24/1/33	Veld Reserve	—	0·18	33·7	—
1046	6/2/33	Veld Reserve	—	0·25	47·6	—
1069	8/5/33	Veld Reserve	—	0·56	60·0	—
1220	29/11/34	Veld Reserve	—	0·87	67·8	—
1595	22/11/35	Veld Reserve	—	0·41	—	—
1219	19/11/34	Railway enclosure Fauresmith	—	1·23	80·74	—
1221	1/12/34	Railway enclosure Fauresmith	—	5·14	89·89	—
1235	28/12/34	Railway enclosure Fauresmith	—	1·48	65·4	—
1240	25/1/35	Railway enclosure Fauresmith	—	0·89	68·82	—
1274	5/3/35	Railway enclosure Fauresmith	—	0·54	35·2	—
815	31/10/32	Tietiespan near homestead	Collected during dikkop outbreak	0·31	46·9	Very strong band 5300–5375, and 5132.
817	31/10/32	Tietiespan near homestead	Collected during dikkop outbreak	0·10	48·0	—
1226A	28/12/34	Dassiespoort yard	Collected during dikkop outbreak Spreading	1·51	68·65	Very strong band 5273–5343, 580–60000.
1227	28/12/34	Dassiespoort yard	Collected during dikkop outbreak. Upright	0·29	66·01	Very strong band 5280–5453, weaker band 5130–5265, 5647–5806, 5885.
1228	28/12/34	Dassiespoort river bank	Spreading	0·52	68·73	—
1229	28/12/34	Dassiespoort river bank	Upright	0·95	63·4	Strong 5160–5300.
1230	28/12/34	Dassiespoort river bank	Wilted	1·03	31·3	—
1234	28/12/34	Dassiespoort garden	Widely spread	1·30	67·4	—
1242	28/1/35	Dassiespoort river bank	—	0·56	66·13	—
1245	28/1/35	Dassiespoort river bank	—	0·49	68·97	—
1243	28/1/35	Dassiespoort garden	—	0·31	58·30	—
1244	28/1/35	Dassiespoort garden	—	0·62	57·16	—
1231	28/12/34	Koksfontein, limestone	Collected during dikkop outbreak	0·18	57·44	5127 and 5230.
1232	28/12/34	Kalabasdrift	—	0·69	65·9	—
1233	28/12/34	Rietriver bridge	—	0·20	62·1	—
1237	22/1/35	Onbekend, Middelburg District	Collected during dikkop outbreak Upright	0·18	37·19	5135; 5831.
1238	22/1/35	Onbekend, Middelburg District	Spreading	0·27	48·0	—
1239	22/1/35	Onbekend, Middelburg District	Widely spreading	0·55	54·94	—
1250	21/1/35	Victoria West	Collected during dikkop outbreak	0·37	8·6*	5212; 5925; 5810.
1251	21/1/35	Victoria West	,, ,, ,,	0·13	9·0*	5035; 5810; 5925.
1252	21/1/35	Victoria West	,, ,, ,,	0·08	8·4*	5315–5420 (very strong); 5142; 5890.
1253	25/2/35	Poortjie	Fresh, spreading	0·85	74·49	—
1254	25/2/35	Poortjie	Collected during dikkop outbreak. Upright	0·34	38·80	5062 very strong; 5200–5300 very strong. 5800–5900.
1255	25/2/35	Poortjie	Collected during dikkop outbreak. Upright, near kopje	0·50	57·19	5175–5290 very strong.
1808	26/3/36	Poortjie	—	0·20	—	—
1785	3/2/37	Poortjie	Fresh, spreading	0·80	74·14	—

* Received here rather dry.

TABLE II.

TRIBULUS.—HCl-figure to see whether cleavage products are present.

Plant Powder extracted with 40% acetone, 90% acetone, ether extract of acetone treated with equal amount of HCl.

No.	Date.	Locality.	Look of plant.	Condition	8 Per cent. HCl.	16 Per cent. HCl.	22 Per cent. HCl.	Conc. HCl.
815	31/10/32	Tietiespan	Upright	Wilted, collected during dikkop outbreak	Faint green colour	Blue green	Blue green	Blue green, (ether from start olive green.
1032	10/12/32	Reserve	Creeping	Wilted	No colour	No colour	Faint blue green	Blue green.
1033	29/12/32	Reserve	Creeping	Wilted	No colour	No colour	No colour	Green.
1034	9/1/33	Reserve	Creeping	Fresh	No colour	No colour	Faint green	Faint green.
1042	24/1/33	Reserve	Creeping	Wilted	No colour	No colour	Very faint green	Darker green.
1046	6/2/33	Reserve	Creeping	Wilted	No colour	No colour	No colour	Faint green.
1069	8/5/33	Reserve	Creeping	Fresh	No material	—	—	—
1219	19/11/34	Railway enclosure	Creeping	Fresh	No colour	No colour	Green	Green.
1220	29/11/34	Reserve	Creeping	Wilted	No colour	Greenish tint	Green	Dark green.
1221	1/12/34	Railway enclosure	Creeping	Fresh	No colour	No colour	Faint green	Green.
1226A	28/12/34	Dassiespoort yard	Creeping	Fresh, collected during dikkop outbreak	No colour	Blue green	Blue green	Dark blue green.
1227	28/12/34	Dassiespoort yard	Upright	„ „	No colour	Tinted	Green	Total green in acid.
1228	28/12/34	Dassiespoort river	Creeping	„ „	No colour	Faint green	Green blue	Green.
1229	28/12/34	Dassiespoort river	Upright	„ „	Faint green	Blue green	Blue green	Blue green.
1231	28/12/34	Koksfontein	Upright	Rather dry, collected during Dikkop outbreak	No colour	Tinted	Faint blue	Blue green.
1232	28/12/34	Kalabasdrift, Ventersvlei	Creeping	Drier than Dassiespoort samples	No colour	No colour	Faint green blue	Faint blue green.
1233	28/12/34	Riet river bridge	Creeping	„ „	No colour	No colour	No colour	No colour.
1234	28/12/34	Dassiespoort garden	Creeping	Very juicy	No colour	No colour	No colour	Faint green.
1235	28/12/34	Railway enclosure	Creeping	Fresh	No colour	No colour	No colour	Faint green-blue.
1237	22/1/35	Onbekend, Middelburg	Upright	Wilted, collected during Dikkop outbreak	Greenish	Light green	Green	Deep green.
1238	22/1/35	Onbekend, Middelburg	Creeping	Less wilted than 1237	No colour	No colour	No colour	Faint green.
1239	22/1/35	Onbekend, Middelburg	Creeping	Fresh	No colour	No colour	Faint green	Blue green.
1240	25/1/35	Railway enclosure	Creeping	Fresh	Faint green	Faint green	Green	Deep green.
1241	28/1/35	Riet river bridge	Creeping	Wilted	No colour	No colour	Pale green	Green.
1242	28/1/35	Dassiespoort river	Creeping	Wilted	No colour	Light green	Light green	Blue green.
1243	28/1/35	Dassiespoort garden	Upright	Wilted	Faint green	Blue green	Blue green	Dark blue green.
1244	28/1/35	Dassiespoort garden	Creeping	Wilted	No colour	Faint green	Blue green	Deep green.
1245	28/1/35	Dassiespoort river	Creeping	Wilted	Light green	Blue green	Blue green	Blue green.
1250	21/1/35	Victoria West	Upright	Wilted, collected during Dikkop outbreak	No colour	Faint green	Green	Green.
1251	21/1/35	Victoria West	Upright	„ „	No colour	No colour	Pale green	Green, has very little chlorophyll.
1252	21/1/35	Victoria West	Upright	„ „	No colour	Faint green	Green	Green.
1253	25/2/35	Poortjie, Fauresmith	Creeping	Fresh	No colour	Just faint tint	Blue green	Dark blue green.
1254	25/2/35	Poortjie, Fauresmith	Upright	Wilted, collected during Dikkop outbreak	Faint tint	Blue green	Dark blue green	Blue green, ether extracted.
1255	25/2/35	Poortjie, Fauresmith	Upright	„ „	No colour	Lightblue green	Dark blue green	Dark blue green, complete ether extracted.
1274	5/3/35	Railway enclosure	Creeping	Fresh	No colour	No colour	Slightly coloured	Blue green.

TABLE III.

Cleavage Products of Chlorophyll. Absorption bands in acid of different percentages.

Slit ½; 0·05 mm. daylight. Depth of layer 12 mm. Extracts of 1 gram dry material.

No.	Date.	Locality.	In 8 per cent HCl. (ether extracted)	In 16 per cent. HCl. (ether extracted).	In 22 per cent. HCl. (ether extracted).
815	31/10/32	Tietiespan........	4900; 5120..	4837–5042; 5193; beyond 4500 and 6500...	4808–5000; 5193; beyond 6300 and 4500 diluted 3 times beyond 6500.
1231	28/12/34	Koksfontein............	Too weak.	Too weak........	4890–5026; 5200; beyond 6550.
1228	28/12/34	Riet river bank...	Too weak..	4835–4985, 5200. 5825; beyond 4500 and 6500	4850–5000; 5184; beyond 6500 and 4500.
1243	28/ 1/35	Dassiespoort garden.. .	4900..	4812–5008; max. 5955; 5200; beyond 6500	4470; 4826–5020; 5182–5424; 5195; 5776; 6416–6400.
1250	21/ 1/35	Victoria West..........	Too weak	Too weak..	Too weak.
1254	25/ 2/35	Poortjie...............	Too weak.... ...	4915–5080; 5120; beyond 6500 . ..	4385; 4824–4997; 5200, beyond 6500.
1255	25/ 2/35	Poortjie...	Too weak... . . .	4852–4965; 5144; 5762; beyond 6500 and 4300	4859–4928; 5162; beyond 4548 and 6500.
1245	28/ 1/35	Dassiespoort river.......	—	—	4855–5048, 5657–5765; and 4590, 5146–5397; 6064–6400; and 6500.
1245	28/ 1/35	Dassiespoort river	—	—	Conc. HCl. 4845–5035; 5159; 5278–5456; 5654–5731; 5731–5891; 6660 end absorption and 4560.

TABLE IV.

Absorption Bands of the Yellow Pigments of Tribulus terrestris.

(Daylight slit 0·05 mm. layer thickness 12 mm., initial material used differs in the single cases, but the end concentration corresponded to ± 2·7 mgm. Carotene in 1000 ccs. which could be diluted still further or evaporated down.)

No.	Date.	State of Plant.	Centres. Adsorption bands 1 and 2 of chromatogram gave the following absorption.	Centres. Adsorption band 3 of chromatogram gave the following absorption.	Centres. Adsorption bands 4 and 5 of chromatogram gave the following absorption.		Ether extract of freshly prepared plant gave the following absorption. bands.
1032	10 12 32	Badly wilted..........	*In Benzine.* 4518; 4834, in CS_2 4855; 5210 probably mostly Carotene	—	*In Benzine.* 4451; 4768 (Guilbert Method) 4483; 4779 (Willst) probably Lutein, but band 4200 not seen)		No absorption beyond 5000.
1034	9/ 1 33	Fresh................	In CS_2 4857; 5189 Carotene In ether 4552; 4853	In CS_2 4500; 4750; 5078; Lutein Flat prisms, red brown, close clusters. Swallow tails on evaporation ex CS_2	In CS_2 4700; 5011 Violaxanthin	—	No absorption beyond 5000.
1042	24 1/33	Wilted................	In alcohol 4525; 4887 Carotene	In alcohol 4458; 4776; 4200 Lutein	In alcohol 4715; 4420; 4175 Violaxanthin	—	No absorption beyond 5000.
			In CS_2 5200; 4855..	In CS_2 4750; 4450; 5077	In CS_2 4400; 4693; 5010	—	—
1046	6/ 2/33	Wilted................	In CS_2 4870; 5180 Carotene mostly	In CS_2 4460; 4754; 5082	(4) In CS_2 4500; 5116; 4787; plate-like prisms	(5) In CS_2 4400; 4678; 5006; very sharp	Absorption towards 5350.
				Thick prisms ex CS_2 Lutein	? Zeaxanthin........	Violaxanthin.........	—
1069	8/ 5/33	Fresh................	In CS_2 4850; 5210..	(3) In CS_2 4460; 4760; 5080	Bds. 3 and 4 were not very sharp Reading difficult. (4)	In CS_2 4400; 4700; 5006	No absorption beyond 5000.
1219	19/11/34	Fresh................	In CS_2 4855; 5210 Carotene	In C 4450; 4741. 5081 Lutein Large long crystals in ether-alcohol	In CS_2 4400; 5000; 4690 Violaxanthin —	There were many bands in this chromatogram and a second and third chromatogram had to be made. These are the final results. For a long time Lutein and Violaxanthin adsorption bands were mixed. But there was no other pigment present, as first thought.	No absorption beyond 5000.
1226A	28/12/34	Fresh................	In benzine 4523; 4831	In benzine (3) 4350. 4580	In benzine (4) 4483; 4782	(5) 4490. 4788.......	No absorption beyond 5060.
			In CS_2 4520; 4830..	In CS_2 4750; 4450. 5080	In CS_2 4400; 4690; 5000 (band very weak)	—	—
1230	28 12 34	Collected during dikkop outbreak, wilted	In CS_2 4520; 4830..	In CS_2 4450; 4750; 5080 Lutein	In CS_2 5200; 4820; 4500 Zeaxanthin	4400; 4690; 5095.. Violaxanthin	Absorption at 5350 and 5570.
1237	22 1 35	Collected during dikkop outbreak, wilted	—	—	—	—	Absorption at 5350 and 5570.
1252	21 1/35	Collected during dikkop outbreak, wilted	In benzine 4517; 4817 Carotene	In benzine 4479 and 4516; 4790 Lutein	In benzine 4504; 4830 Zeaxanthin	—	Absorption 5350 and 5570.
1253	25/1 35	Spreading, but wilted ...	—	—	—	—	Absorption 5350; 5570; 5090.
1254	25/2 35	Wilted, upright........	—	—	—	—	Absorption 5350; 5570.
1255	25/2/35	Wilted, upright.........	In CS_2 5210; 4855 Carotene	4450; 4750; 5080 Lutein	5200; 4830; 4500 Zeaxanthin	—	Absorption 5350; 5570.

TABLE V.

Carotinoids of Tribulus terrestris from different localities and under different conditions.

No.	Date.	Locality.	Condition of Plant	Chlorophyll in percentage of dry matter	Total yellow pigments in percentage of dry matter	Ratio of green to yellow pigments.	Xanthophyll in percentage of dry matter.	Carotene in percentage of dry matter.	Remarks.
1032	10 12 32	Veld Reserve..	Badly wilted......... ..	0.38	0.0194	19.5	0.0095	0.0099	—
1042	24 1 33	Veld Reserve..	Wilted	0.18	0.0026	69.8	0.0010	0.0016	—
1069	8 5, 33	Veld Reserve ..	New plants fresh, green	0.56	0.0247	16.1	0.0247	0.0 00	—
1220	29 11 34	Veld Reserve	Plant not as fresh as from other places	0.08	0.0055	14.5	0.0029	0.0026	—
1595	22 11 35	Veld Reserve............	Spreading..	0.41	0.041	10.0	0.0262	0.0146	—
1226A	28 12 34	Dassiespoort yard...	Fresh..	1.51	0.034	44.4	0.0270	0.0070	Small flower.
1229	28 12 34	Dassiespoort river bank....	Fresh	0.95	0.0143	66.0	0.0090	0.0053	Medium flower.
1231	28 12 34	Koksfontein, lime stone formation	Rather dry eaten by locusts	0.18	0.0078	23.0	0.0043	0.0035	No flower.
1233	28 12 34	Riet river bridge..	Rather dry..	0.20	0.0277	7.20	0.0171	0.0106	Small flower.
1237	22 1 35	Onbekend, Middelburg District..	Collected after dikkop outbreak, very wilted	0.18	0.0158	10.1	0.0099	0.0059	Upright small flower.
1238	22 1 35	Onbekend, Middelburg District .	Better than 1237... .	0.28	0.0210	13.3	0.0142	0.0068	Prostrate.
1239	22 1 35	Onbekend, lands	Fresh	0.55	0.0096	57.3	0.0056	0.0040	Widely spreading
1250	21 1 35	Victoria West.	Collected during dikkop outbreak wilted	0.37	0.0237	15.6	0.0151	0.0086	Upright.
1251	21 1 35	Victoria West...	,, ,,	0.13	0.0044	28.8	0.0017	0.0027	Upright.
1254	25 2 35	Poortjie	,, ,,	0.34	0.048	7.08	0.030	0.018	Upright.
1808	26 3 35	Poortjie.	,, ,,	0.20	0.0056	35.5	0.0033	0.0023	—
1785	3 2 37	Poortjie.........	Fresh	0.80	0.044	18.3	0.026	0.0186	Spreading.
815	31 10 32	Tietiespan	Collected during dikkop outbreak, very wilted	0.31	0.0073	42.4	0.0033	0.0040	Upright.

TABLE VI.

TRIBULUS.

No.	Date.	Locality.	NO_3 content. Determined. Per 100 gm. fresh matter.	NO_3 content. Calculated. Per 100 gm. dry matter.	NO_3 content. Determined. Per 100 gm. dried material.	NO_3 content after treating with 0·15 gm. aspartic acid. Determined. Per 100 gm fresh matter	NO_3 content after treating with 0·15 gm. aspartic acid. Determined. Per 100 gm dry matter.	Difference expressed as NO_3 probably nitrite. Per 100 gm fresh matter.	Difference expressed as NO_3 probably nitrite. Per 100 gm. dry matter	Water content	Remarks.
815	30/10/32	Tietiespan......	0.41	—	0.476	0.194	0.365	0.206	0.111	46.9	Collected during dikkop outbreak, wilted.
816	30/10/32	Tietiespan.......	0.30	—	0.479	0.209	0.459	0.081	0.020	54.5	Fresh.
817	30/10/32	Tietiespan......	0.381	—	0.734	—	0.574	—	0.160	48.0	Collected during dikkop outbreak, wilted.
—	8/12/32	Dassiespoort	—	After alcohol extract. —	0.167	—	0.049	—	0.118	43.2	Wilted.
1032	10/12/32	Veld Reserve ..	0.395	—	—	0.275	—	0.120	—	42.3	Wilted.
1033	29/12/32	Veld Reserve .	—	—	0.977	—	0.738	—	0.239	40	Wilted. Strong reaction for nitrite
1034	9/1/33	Veld Reserve. .	0.113	0.167	0.167	0.115	0.161	0.0	0.006	60.2	Fresh no reaction for NO_3.
1042	24/1/33	Veld Reserve....	0.145	0.219	—	—	—	—	—	33.7	Wilted, Strong reaction for nitrite.
1046	6/2/33	Veld Reserve.	0.054	—	0.099	—	—	—	—	47.6	Wilted. Strong reaction for nitrite.

Section V.

Toxicology.

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On the Constitution of the Bitter Principle "Geigerin" I. The Isolation of Various Degradation Acids.

By H. L. DE WAAL, Section of Pharmacology and Toxicology, Onderstepoort.

(Read at Onderstepoort on 8th September, 1937.)

Introduction.

According to the properties of the bitter principle Geigerin and the active principle Vermeeric acid of the "Vermeerbos" (*Geigeria aspera*, Harv.), described by Remington and Roets (1936) and Rimington, Roets and Steyn (1936), these substances would appear to be closely related with regard to their chemical structures. In summarizing the results already obtained, these authors suggest part formulae for Geigerin (1), Vermeeric acid (2) and its dilactone Vermeerin (3) as follows, giving an indication of the possible involvement of one atom of oxygen in a keto-enol tautomerism:

(1) $C_{15}H_{20}O_4$:

$$C_{14}H_{20}O \left\{ \begin{array}{l} -CO \\ \quad | \\ -O \\ \\ =O \end{array} \right. \quad \text{or} \quad C_{14}H_{19} \left\{ \begin{array}{l} -CO \\ \quad | \\ -O \\ \\ -OH \rightleftharpoons =O + H \text{ to ring.} \end{array} \right.$$

(2) $C_{18}H_{28}O_7$:

$$C_{11}H_{24} \left\{ \begin{array}{l} -COOH \\ -COOH \\ -OH \\ -OH \\ =O \end{array} \right. \quad \text{or} \quad C_{16}H_{24}O \left\{ \begin{array}{l} -COOH \\ -COOH \\ -OH \\ -OH \rightleftharpoons =O + H \text{ to ring.} \end{array} \right.$$

(3) $C_{18}H_{24}O_5$:

$$C_{16}H_{24}O \left\{ \begin{array}{l} -\,CO \\ \quad | \\ -\,O \\ \\ -\,CO \\ \quad | \\ -\,O \end{array} \right.$$

These substances being in all probability closely related to one another, constitutional work was begun on the chemical constitution of the bitter principle Geigerin. Should the structure of the latter be elucidated, then similar methods of constitutional investigation when applied to Vermeeric acid or Vermeerin, might be expected to lead successfully to the clearing up of their structures also. Meanwhile it may be pointed out that the bitter principles, although very interesting subjects for investigation have proved to be most difficult from the constitutional structural point of view. Thus Beth (1936) describing a few scores of bitter principles already isolated from different plants, especially the families Gentianaceae, Compositae and Labiateae points out that up till then not a single bitter principle's structure had been fully elucidated.

In this article we wish to record the first results already obtained on the constitution of Geigerin and to describe various degradation products obtained by oxidation or otherwise. These have been very encouraging. Until more starting material has however been prepared and until further results have been obtained, we do not wish to enter into a detailed discussion of its possible structural formula at this stage.

THE APPLICATION OF THE LEGAL TEST AS A METHOD FOR DETECTING ACETALDEHYDE IN GEIGERIN OXIDATION EXPERIMENTS.

According to Meyer (1931, p. 446) a characteristic intense colouration is obtained when to solutions of ketones or aldehydes preferably in water (also alcohol or ether) 0·5 to 1 c.c. of a freshly prepared 0·3 to 0·5 per cent. sodium nitroprusside solution is added followed by alkalinification with concentrated potassium hydroxide (D = 1·14).

In the aliphatic series this reaction is always positive when the group attached to the ketonic or aldehydic radicle (in α-position) consists of carbon and hydrogen only. Aldehyde or ketone groups directly bound to a ring or included in a ring structure do not give the reaction, neither do ortho-aldehydic acids.

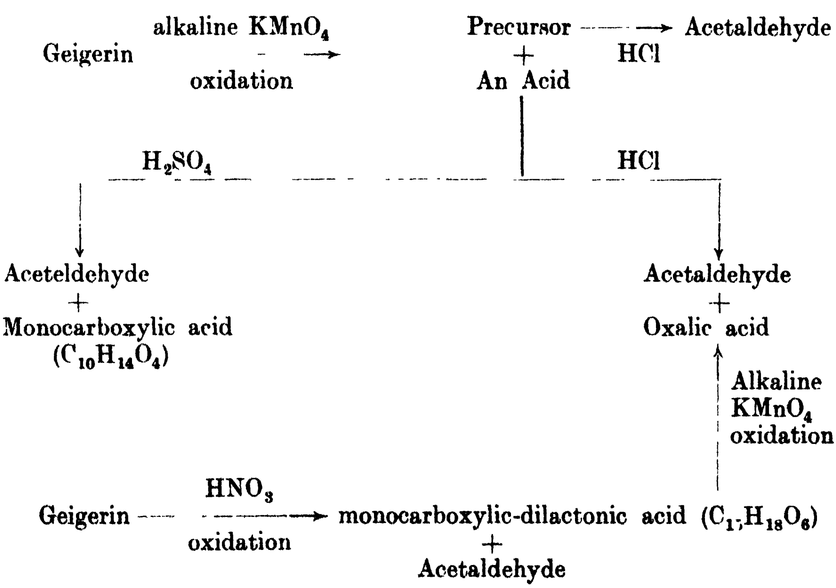
The colours produced vary from orange-red, red to violet-red, which on the addition of organic acids change from red to indigo-blue or from violet blue to blue-green e.g.

The solid α- or β-Geigerin is not a ketone, nor does it show aldehydic or ketonic properties in a neutral solution (it is presumably present in the enol-form) but in dilute hydrochloric acid solution it reacts as a ketone. The Legal test is therefore negative with Geigerin. When Geigerin is dissolved in a 10 per cent. hydrochloric

acid solution (ketonic-form), cooled down and then neutralized with 16 per cent. potassium hydroxide the immediate application of the Legal test yields a negative result. This shows either (1) that the enolic form is again obtained on neutralization or (2) that although Geigerin may still be present in the ketonic form, that the carbonyl-group is directly contained in a ring structure or immediately attached to it.

In the oxidation processes described below (see experimental part) acetaldehyde is very frequently formed as a byproduct during the oxidation. Under certain circumstances the acetaldehyde is most probably liberated from a pre-cursor formed during the oxidation and can thus only be detected after acidification. Under other conditions (ether extractions of acid mediums or acid oxidations) acetaldehyde accompanies the main oxidation product formed as a sticky oily material. When the latter is washed away with pure ether (only Merck's and Schering-Kahlbaum's absolute ether has been used in these extractions), the acetaldehyde may be easily and very conveniently detected by means of the Legal test. The ethereal solution or a watery or even an absolute alcoholic solution (when acetadehyde is present) will immediately give a positive test with the Legal reagent, the watery phase turning intense orange-red. The usual identification tests e.g. its pungent odour, its 2-4 dinitrophenylhydrazone derivative with Brady's reagent and other tests will confirm its presence. Blank experiments with pure ether, absolute alcohol or water will give only the "blank" greenish-yellow colouration.

The following diagram will demonstrate the convenience of the Legal test for detecting the acetaldehyde also formed during the isolation of the various oxidation products (compare the experimental part):



Neither of the degradation acids, $C_{10}H_{14}O_4$ (M.P. 201°) and $C_{15}H_{18}O_6 \cdot H_2O$ (M.P. 280°) give the Legal test; thus in solution the application of the Legal test will quickly affirm the presence or absence of acetaldehyde.

Experimental.

Improved Geigerin Extraction.

About 75 Kilogram of dried ground " Vermeerbos " was extracted in the following way:

3·5 Kg. dried material was extracted with ether in a large soxhlet-type apparatus for 3 working days (i.e. about 22 hours). The ether was then drawn off, amounting to about 8 litres in volume. The ether solution was then divided into two equal portions and each part shaken with about 5 litres of 1 per cent. hydrochloric acid (in 5 or 6 portions), i.e. a total volume of 10 litres. Through this solution air was then drawn for 3 to 4 hours when the solution has a greenish-yellow tint with a pleasant ether-free fruity smell. The solution was then shaken with a little charcoal, filtered and now shaken with 8 lb. of chloroform (in successive portions). The chloroform solution was then washed with a one per cent. sodium carbonate solution, becoming nearly colourless, and again washed with distilled water.

The chloroform solution was then dried over sodium sulphate, concentrated at reduced pressure to about 500 c.c. of chloroform and left to evaporate in front of a fan. After 2 to 3 days the resulting crystalline residue was boiled out with 300 to 400 c.c. of distilled water and filtered into open dishes. Crystallization immediately set in on cooling and after recrystallization from water a yield (on the average) of 1·5 gm. of Geigerin was obtained, i.e. 0·43 gm. per 1 Kgm. dried plant material.

Oxidation of Geigerin with Nitric Acid to a monocarboxylic-dilactonic acid ($C_{15}H_{18}O_6 \cdot H_2O$).

Two different methods both yielding good results, are described below:—

(*a*) 1 gm. Geigerin and 50 c.c. nitric acid (1:1) were refluxed for 12 hours, during which time nitrogen peroxide fumes were evolved finally yielding a pale yellow solution. On cooling it was diluted with distilled water and then thoroughly shaken 3 times with chloroform, the chloroform solution washed once with water, dried over exsiccated sodium sulphate and then evaporated in front of a fan at room temperature. Crystals separated practically immediately. After the solution had been evaporated to dryness, the crystals were washed with ether and were obtained as colourless hexagonal prisms with a melting-point, crude, of about 250° and in a yield of 0·25 gm.

The ether washings evaporated at room temperature left acetaldehyde.

The original solution was again shaken 3 times with chloroform and after the same treatment yielded another 50 mgm. of the new product, thus increasing the yield to about 300 mgm. The material

was twice recrystallised from water. The crystals turned opaque * at 105°; above 240° hexagonal prisms shot out which melted sharply at 280* (corr.) without decomposition.

The same oxidation product can be obtained by the oxidation of Geigerin with nitric acid (1 : 3 and 1 : 2), concentrated nitric acid (D = 1·4) and fuming nitric acid. The following method is especially recommended:—

(*b*) Introduce into an open pyrex dish (capacity about 50 c.c.) 1·5 gm. Geigerin and cover the substance with about 20 c.c. of concentrated nitric acid (D = 1·4) and warm on a boiling waterbath. The crystals easily dissolve. The oxidation is continued for about one day (8 hours). Continue the addition of concentrated nitric acid now and then and finally allow to concentrate to about 10 c.c. Allow to cool down (crystals frequently separating immediately) dilute with water to about 100 c.c. and leave to stand in an ice-chest overnight. Recrystallised from water 400 mgm. of the pure oxidation acid is obtained (M.P. 280—see Fig. 1) (a byproduct of the oxidation is acetaldehyde).

Fig. 1.—Acid obtained by the nitric acid oxidation of Geigerin, m.p. 280°, ×25.

Properties.—The substance—

(1) is easily soluble in ammonia, alkalis and dilute sodium carbonate solution—is therefore an acid—and may be recovered on the addition of dilute acids;

(2) dissolves slowly without colour in concentrated sulphuric acid but is practically insoluble in dilute mineral acids;

(3) is insoluble in benzene, ether and petroleum ether;

(4) is sparingly soluble in chloroform and ethyl acetate;

(5) is soluble in water, methyl alcohol and ethyl alcohol;

(6) is readily soluble in acetone;

(7) does *not* react with Brady's reagent;

(8) does *not* give a Legal test;

(9) gives no colour with dilute or concentrated hydrochloric acid.

* Kofler micromelting point apparatus.

*Micro-analysis.**

(1) Dried at room temperature in high vac. over P_2O_5.

(*a*) 5·208 mgm.: 10·960 mgm. CO_2; 3·060 mgm. H_2O.

found C = 57·41; H = 6·58.

Calc. C = 57·68; H = 6·46.

$C_{15}H_{18}O_6 \cdot H_2O$
(312·31)

(*b*) 0·201 mgm. in 3·756 mgm. Camphor: $\triangle = 6 \cdot 7$
i.e. mol. weight = 319 (calcd. 312·31).

(2) Dried at 110° C. in highvac.

(*a*) 5·324 mgm.; 11·945 mgm. CO_2; 2·920 mgm. H_2O.

(*b*) 4·539 mgm.; 10·230 mgm. CO_2; 2·550 mgm. H_2O.

found (*a*) C = 61·22; H = 6·14.

,, (*b*) C = 61·49; H = 6·29.

Calc. C = 61·22; H = 6·17.

$C_{15}H_{18}O_6$.

Micro-titration.—16·6 mgm. of the acid dissolved in about 10 c.c. water required 0·65 c.c. 0·1 N NaOH, i.e. assuming one –COOH the molecular weight is 299.

Rotation.—Weight = 59·8 mgm.

Volume = 15·0 c.c. abs. alc.

$$\Theta = +1 \cdot 45^\circ.$$

$$\text{Therefore } \left[\alpha\right]^{25}_{D} = \frac{+1 \cdot 45 \times 100 \times 15}{2 \times 5 \cdot 98}$$

$$= +181 \cdot 85^\circ.$$

Methyl-ester.

To a solution of 50 mgm. of the above oxidation acid dissolved in about 6 to 7 c.c. of acetone, an ethereal solution of diazomethane was slowly added (ice-cooling) until a slight excess of diazomethane was noticeable. The solution was then left to stand overnight well-stoppered. It was then allowed to evaporate and the crystalline residue (needles grouping in aggregates) was recrystallized from dilute methyl alcohol. After recrystallization,, washed with 96 per cent. alcohol and then with ether, the colourless six-sided prismatic needles melted sharply without decomposition at 190-191° (corr.).

Micro-analysis.

4·974 mgm.: 11·325 mgm. CO_2; 2·970 mgm. H_2O; 0·010 mgm. res.

found C = 62·25; H = 6·70.

Calc. C = 62·33; H = 6·54.

$C_{14}H_{17}O_4COOCH_3$:
($C_{16}H_{20}O_6$)

* All micro-analysis by Dr. Ing. A. Schoeller, Berlin.

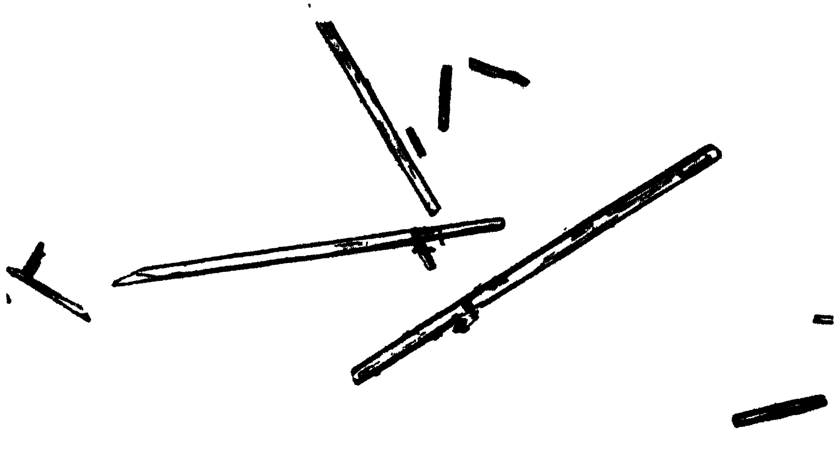


Fig. 2.—Methyl ester of monocarboxylic dilactonic acid m.p. 190-191°, ×50.

Presence of Lactone groupings in the "new" acid, m.p. 280°.

32·0 mg. of the acid was dissolved in the cold in 5 c.c. of 96 per cent. alcoholic potassium hydroxide (F 0·097) and after five minutes the solution back titrated with 0·1 N hydrochloric acid.

5 c.c. Alcoh. KOH (F 0·097)... = 4·85 c.c. of 0·1 N KOH.

Back titration required ... . 2·90 c.c. of 0·1 N HCl.

Therefore difference . . . . 1·95 c.c. 0·1 N KOH

Now 31·23 (mol. wt. 312·3) mgr. acid req.: 1 c.c. 0·1 N KOH for one - COOH.

Therefore 32·0 mgr. acid reqd.: 1·024 c.c. 0·1 N KOH for one - COOH.

i.e. theory for 2 carboxyles = 2·05 c.c. 0·1 N KOH.

found 1·95 c.c. 0·1 N KOH.

To the above solution 4·85 c.c. 0·1 N alcoholic potassium hydroxide was again added and the solution left for 5 hours at 37° C.

Back titration required 3·95 c.c. 0·1 N HCl.

Therefore difference ... 0·90 c.c. 0·1 N KOH.

Theory for one - COOH = 1·02 c.c. 0·1 N KOH.

Again 4·85 c.c. of 0·1 N alcoholic potass. hydroxide was added and the solution left for another 5 hours at 37°.

Back titration required 4·90 c.c. 0·1 N HCl.

Therefore no significant difference.

These figures indicate that after 5 minutes one lactone group is saponified to a carboxylic group and after 5 hours a second lactone group is saponified to a second carboxylic group. Finally we thus have a tribasic acid (the original acid being monocarboxylic) which was again proved by the relactonization.

Relactonization of the Tricarboxylic Acid.

Continuing with the above solution containing the tricarboxylic acid, experiments were conducted to determine whether any of the carboxyle groups could relactonise.

5 c.c. 0·1 N HCl was added and the solution left for 20 hours at 37° C;

Back titration required	2·80 c.c. 0·1 N KOH.
Therefore difference ...	=2·20 c.c. 0·1 N HCl.
Theory for 2 lactones ...	=2·05 c.c. 0·1 N HCl.

After the addition of another 2·0 c.c. 0·1 HCl and leaving the solution for 6 hours at 37°, it was back titrated and the back titration required exactly 2 c.c. of 0·1 N KOH; therefore no change.

Thus 2 of the 3 carboxyl groups were relactonized after 20 hours.

Duplicate Saponification and Re-lactonization.

32·0 mgr. of the dilactonic-monocarboxylic acid (M.P. 280°) was dissolved in 5 c.c. of 0·1 N alcoholic KOH at room temperature and back titrated with 0·1 N HCl after seven minutes.

Back titration required 2·80 c.c. 0·1 N HCl.

Therefore difference =2·20 c.c. 0·1 N KOH.

After another hour (i.e. one hour and seven minutes) a back titration of 5 c.c. N alcoholic KOH with 0·1 N HCl gave a difference of 0·80 c.c. 0·1 N KOH.

Therefore total difference =3·00 c.c. 0·1 N KOH.

Theory for 3 – COOH = 3·07 c.c. 0·1 N KOH.

After 18 hours the total difference was raised to 3·15 c.c. 0·1 N KOH, i.e. a practically complete saponification of the monobasic-dilactonic acid was accomplished after 67 minutes to a tribasic-acid.

Similarly relactonization of the above solution took place as follows:—

After 30 minutes difference	=0·80 c.c. 0·1 N HCl.
After 2 hours difference ...	=1·30 c.c. 0·1 N HCl.
After 7 hours difference ...	=1·60 c.c. 0·1 N HCl.
After 24 hours difference ...	=1·80 c.c. 0·1 N HCl.
Theory for 2 lactone-groups	=2·05 c.c. 0·1 N HCl.

Summarising these results we have for the monocarboxylic-dilactonic-acid (M.P. 280°) an empirical formula of $C_{15}H_{18}O_6 \cdot H_2O$ constituted as follows:—

$$\begin{array}{c} C_{12}H_{17} \\ \cdot H_2O \\ (I) \end{array} \left\{ \begin{array}{l} -COOH \\ -CO \\ \;\;| \\ -O \\ -CO \\ \;\;| \\ -O \end{array} \right. \xrightarrow{+\ CH_2N_2} C_{12}H_{17} \left\{ \begin{array}{l} COOCH_3 \\ -CO \\ \;\;| \\ -O \\ -CO \\ \;\;| \\ -O \end{array} \right.$$

$$\begin{array}{c} C_{12}H_{17} \\ \cdot H_2O \\ (I) \end{array} \left\{ \begin{array}{l} -COOH \\ -CO \\ \;\;| \\ -O \\ -CO \\ \;\;| \\ -O \end{array} \right. \xrightarrow{+\ \text{aqueous KOH}} C_{12}H_{17} \left\{ \begin{array}{l} COOK \\ -CO \\ \;\;| \\ -O \\ -CO \\ -O \end{array} \right.$$

$$\begin{array}{c} C_{12}H_{17} \\ \cdot H_2O \\ (I) \end{array} \left\{ \begin{array}{l} -COOH \\ -CO \\ \;\;| \\ -O \\ -CO \\ \;\;| \\ -O \end{array} \right. \xrightarrow[\substack{\text{(within 5 minutes) at} \\ \text{room temperature}}]{+\ \text{alcoholic KOH}} C_{12}H_{17} \left\{ \begin{array}{l} -COOK \\ -COOK \\ -OH \\ -CO \\ \;\;| \\ -O \end{array} \right.$$

$$\begin{array}{c} C_{12}H_{17} \\ \cdot H_2O \\ (I) \end{array} \left\{ \begin{array}{l} -COOH \\ -CO \\ \;\;| \\ -O \\ \;\;CO \\ \;\;| \\ -O \end{array} \right. \xrightarrow[\text{(approx. 5 hours) at } 37^\circ]{+\ \text{alcoholic KOH}} C_{12}H_{1} \left\{ \begin{array}{l} -COOK \\ -COOK \\ -COOK \\ -OH \\ -OH \end{array} \right.$$

and the relactonization

$$C_{12}H_{17} \left\{ \begin{array}{l} -COOH \\ -COOH \\ -COOH \\ -OH \\ -OH \end{array} \right. \xrightarrow[\text{(1–2 hours) at } 37^\circ]{+\ HCl} C_{12}H_{17} \left\{ \begin{array}{l} -COOH \\ -COOH \\ -OH \\ -CO \\ \;\;| \\ -O \end{array} \right.$$

$$C_{12}H_{17}\begin{cases}-\mathrm{COOH}\\-\mathrm{COOH}\\-\mathrm{COOH}\\-\mathrm{OH}\\-\mathrm{OH}\end{cases} \xrightarrow[\text{(approx. 20 hours) at }37^\circ]{+\ \mathrm{HCl}} C_{12}H_{17}\cdot H_2O\begin{cases}-\mathrm{COOH}\\-\mathrm{CO}\\ \quad |\\-\mathrm{O}\\-\mathrm{CO}\\ \quad |\\-\mathrm{O}\end{cases}$$

(Same as I above).

Considering the ease with which one carboxyl group is relactonised in the presence of 0·1 N hydrochloric acid, it is interesting to compare this phenomenon with that of Vermeeric acid (see Rimington, Roets and Steyn, pp. 517-518). In the latter acid one carboxyl group similarly lactonises much more readily than the second (e.g. in the presence of the hydrochloric acid of the Brady's reagent when the 2·4-dinitrophenylhydrazone of Vermeeric acid is prepared). A possible structural explanation for this phenomenon is discussed further below.

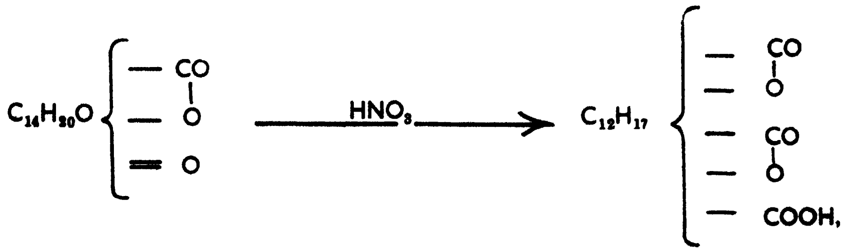
An attempt to prepare a semicarbazone from this new acid was negative. 50 mgm. acid was dissolved in 3c.c. absolute alcohol and to this a solution of 50 mgm. semicarbazide-hydrochloride and 50 mgm. sodium acetate in 0·5 c.c. water was added. The mixture was refluxed for 8 hours. The original acid of M.P. 280° was regained quantitatively.

An acetylation attempt proved also the absence of free hydroxyl groups. 50 mgm. acid dissolved in 1 c.c. of pyridin was left to stand for several days with 0·2 c.c. of acetic anhydride. The original acid of M.P. 280° was again recovered.

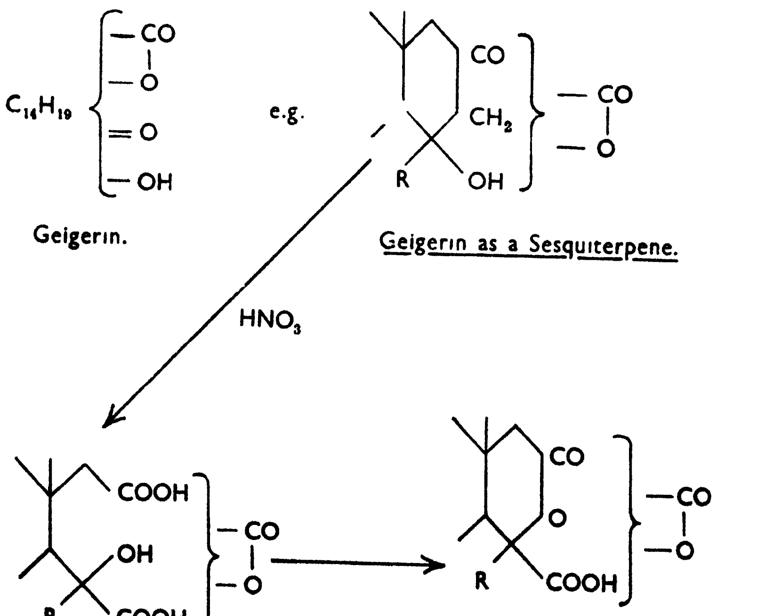
These negative experiments for a carbonyl- or hydroxyl radicle are in harmony with the saponification and relactonization findings above. The nature of the acid—a monobasic-dilactonic acid—accounts for the functions of all its six oxygen atoms. As this acid results from the oxidation of Geigerin ($C_{15}H_{20}O_4$), of which the function of the fourth oxygen atom (see page 395) is still unknown, it may prove very valuable for determining the function of that fourth oxygen atom. Also by comparing the two formulae of Geigerin ($C_{15}H_{20}O_4$) and of the monocarboxylic-dilactonic-acid ($C_{15}H_{18}O_6$) we find that the nitric acid oxidation led to a gain of two oxygen atoms accompanied by a loss of *two* hydrogen atoms only. A review of the literature showed that this acid is still unknown, and further experiments as regards its structure will have to be performed.

Meanwhile we could explain such an oxidation as follows:

The fact that Geigerin, which is a mono-lactone and which is in acid medium mono-ketonic, can be oxidised with nitric acid to a dilactonic-monobasic acid thus



seems to support the view that the fourth oxygen atom in Geigerin is not ether-bound or cyclic, but most likely present in a hydroxyl-group, which cannot be acetylated (steric hindrance). We could then explain such an oxidation by postulating a mechanism similar to that frequently encountered in the camphor group (terpenes). As an an example the oxidation may be cited of camphor by nitric acid through camphoric acid to camphanic acid (Bredt). In an analogous manner the oxidation of Geigerin could be formulated as follows:



Geigerin. Geigerin as a Sesquiterpene.

Dilactonic-monocarboxylic acid.

In the latter acid the lactone group (outside) may be easily saponified (same as in Geigerin where it gives Geigeric acid) and the new-formed lactone group (inside) may be more difficultly

saponified, i.e. as we have found, may be the lactone group which is only saponified after standing for 24 hours (at least) with alcoholic potassium hydroxide at 37°C.

Other facts which seem to coincide with a sesquiterpene structure for Geigerin are (1) the formation of naphthalene on distillation with zinc dust (compare Rimington and Roets p. 497) and (2) the formation (under proper conditions) of a $C_{10}H_{14}O_4$ acid, when Geigerin is oxidised with alkaline permanganate solution. We have again been able to confirm the formation of this acid (compare Rimington and Roets, p. 502) and its properties seem to point to a terpene-acid e.g. camphanic acid (which however still awaits identification).

Judging from the properties of the monocarboxylic-dilactonic acid (M.P. 280°), it appears to be a very stable compound as the following experiment will also serve to exemplify.

To 60 mgm. of the acid dissolved in 10 c.c. glacial acetic acid was added 2 c.c. concentrated sulphuric acid and the mixture warmed on a boiling waterbath (vertical tube) for 2 to 3 days. The acetic acid was distilled off under reduced pressure to a very small volume. The dark brown solution was diluted with a little water and when put into the ice-chest the original acid again crystallised out quantitatively. After recrystallization it had M.P. 280°

Permanganate Oxydation of the Monocarboxylic-dilactonic acid to oxalic acid.

100 mgm. of the above acid (M.P. 280°) and 1 gm. sodium carbonate were dissolved in 5 c.c. of distilled water and 25 c.c. of a 3 per cent. potassium permanganate solution slowly added. The solution was kept gently boiling under a " Normal-Schliff " reflux condensor for 2 hours. After having cooled down, the manganese dioxide of the completely decoloured solution was filtered off.

The still alkaline solution was thoroughly shaken with pure ether. On evaporation the ether left a very small amorphous residue, which on acidification with dilute hydrochloric acid yielded acetaldehyde (2, 4 dinitrophenyl-hydrazone derivative with Brady's reagent of M.P. 160-161 and other identification tests).

The above solution was then acidified with HCl and again thoroughly shaken with ether. On the evaporation of the ether a crystalline substance (shining plates) deposited, which darkened at 60-80°, and melted with sublimation and much evolution of gas at about 150°. The crystals were dissolved in a little cold water and the solution left to evaporate on a watch-glass. They darkened at 70-90°, sublimation occurred from 155° upwards and with gas-evolution the crystals melted at 165°. The substance dissolved with strong effervesence in dilute sodium carbonate solution and was found to be identical with the degradation acid obtained by the permanganate oxidation of Geigerin (see below) and both again identical with oxalic acid.

Degradation of Geigerin to Oxalic Acid.

To 1·5 gm. Geigerin dissolved in 150 c.c. of water was added 50 c.c. of a 10 per cent. sodium carbonate solution. The flask, containing this solution, was fitted with a " Normal-Schliff " reflux condensor, the top end of which again carried a ,, Normal-Schliff " dropping funnel. Through the dropping funnel 180 c.c. of a 2·5 per cent. potassium permanganate solution was dropped in, while the flask was held on a boiling waterbath for two hours. Boiling was then continued on a wire gauze for another hour, during which time another 70 c.c. of the 2·5 per cent. potassium permanganate solution was dropped in—in all therefore 250 c.c. of 2·5 per cent. $KMnO_4$ solution. At this stage decolourisation was very slow and the oxidation was interrupted after 3¼ hours.

When cool the solution was filtered and the faintly yellow clear solution extracted with pure ether in a bubble-extractor for 72 hours. (Except for a little acetaldehyde nothing else could be isolated from the faint residue obtained after the evaporation of the ether).

The solution was then acidified with hydrochloric acid and again extracted in a bubble-extractor with pure ether for several days until complete exhaustion.

After 24 hours extraction, the ether extract on evaporation deposited about 150 mgm. of degradation acid. After another 24 hours, another 80 mgm. were obtained. After one week's continued extraction no more of the degradation acid could be obtained and the total yield amounted to about 400 mgm.

On the evaporation of these ether extracts crystals separated which possessed the following properties:

(1) Soluble without colour in dilute or concentrated HCl.

(2) Soluble without colour in concentrated H_2SO_4.

(3) Soluble without colour in dilute or concentrated NH_4OH.

(4) It dissolves with strong effervescence in 2½ per cent. sodium carbonate solution.

(5) No reaction with Brady's reagent.

(6) Very readily soluble in water.

(7) Easily soluble in absolute alcohol, dilute alcohol, methyl alcohol and acetic acid.

(8) Soluble in ethyl acetate, ether, acetone and toluene.

(9) Practically insoluble in benzene, petroleum ether and carbon tetrachloride.

(10) Soluble in acetic anhydride and dioxan.

From a mixture of acetone and carbon tetrachloride colourless shining plates crystallised, which turned opaque (loss of water) at 75 to 80°, sublimation started at 140° and the crystals then melted at 180°.

Micro-analysis:

4·840 mg. dried at room temperature in h.v. lost 2·628 mg. in weight.

2·723 mg. dried at room temperature in h.v. lost 1·513 mg. in weight.

(1) 2·212 mgm.: 2·090 mgm. CO_2; 0·580 mgm. H_2O.

(2) 0·207 mgm. in 2·748 mgm. Camphor; $\triangle = 31 \cdot 7$, i.e. mol. weight = 95.

(3) 0·254 mgm. in 4·229 mgm. Camphor; $\triangle = 26 \cdot 9$, i.e. mol. weight = 89.

Found: C = 25·77 per cent.; H = 2·96 per cent.; mol. wht. 89 and 95.

$C_2H_2O_4$: calculated: C = 26·67 per cent.; H = 2·22 per cent. mol. wht. = 90.

The analysis and the properties proved that the degradation acid obtained above was identical with oxalic acid. Similarly the degradation acid obtained by the potassium permanganate oxidation of the monocarboxylic dilactonic acid (see p. 14) was identical with oxalic acid in all respects. Again these two degradation acids were fully identical between each other (no melting point depressions etc.).

Attempts to degrade Geigerin with concentrated sulphuric acid.

About 200 mgm. of Geigerin was dissolved in about 10 c.c. concentrated sulphuric acid. The solution was gently warmed to about 50° and the lemon-yellow solution was then slowly run into 100 c.c. of water under continuous stirring so that the temperature did not rise over 80° C (compare Pfeiffer and de Waal (1935).

The pale yellow solution was then shaken with ether and on evaporation of the ether a big crop of colourless crystalline columns separated. The substance proved to be non-phenolic, non-acidic and was easily identified as β-Geigerin (compare Rimington and Roets page 489).

On repeating the above experiment with 1gm. of Geigerin and 40 c.c. of concentrated sulphuric acid run into about 400 c.c. of water (temperature 70-80°C) an ethereal extract of the solution gave a mixture of α- and β-Geigerin only. Repeated extraction with chloroform gave the same results.

Concentrated sulphuric acid is therefore incapable of decomposing Geigerin.

An attempt to oxidise 450 mgm. Geigerin dissolved in about 20 c.c. water and 10 c.c. 96 per cent. alcohol with 4 c.c. of 30 per cent. hydrogen peroxide and refluxing the mixture for 6 hours failed. Unchanged Geigerin was recovered quantitatively.

200 mgm. Geigerin was dissolved in about 20 c.c. glacial acetic acid; 2 c.c. of 48 per cent sulphuric acid was added and the solution heated on a boiling waterbath under a reflux condenser for 3 hours.

The acetic acid was then distilled off at reduced pressure leaving a final volume of about 3 c.c. (dark brown liquid). Then 40 c.c. water was stirred in yielding an oily precipitate which in the course of a fortnight slowly changed into a colourless group of crystals, which after recrystallization from water was found to be a mixture of α- and β-Geigerin.

Attempts to prepare acid amides of Geigerin and the mono-carboxylic-dilactonic acid (M.P. 280°).

Hansen (1931) describes the preparation of Alantolic acid amide from the bitter principle Alanto-lactone by means of cold saturated alcoholic-ammonia solutions. Similar attempts to obtain the acid amide of the lactone, Geigerin, proved negative as the following experiments will illustrate:—

(1) To 10 mgm. of Geigerin 1 c.c. of cold saturated methyl-alcoholic ammonia was added and the solution allowed to stand for one week at room temperature. Unchanged Geigerin of M.P. 189-190° was recovered (compare Abderhalden, 1936) where Alanto-acetamide crystallised out after standing for 2 hours).

(2) To 10 mg. of Geigerin 1 c.c. of cold saturated ethyl-alcoholic ammonia was added and the solution, well stoppered, allowed to stand for 7 days. Unchanged α-Geigerin of M.P. 190°C was then quantitatively recovered.

(These experiments will be repeated with Geigeric acid).

(3) To 10 mg. of the monocarboxylic-dilactonic acid (see p. 398) 1 c.c. of cold saturated methyl-alcoholic ammonia was added and the solution allowed to stand for one week. Unchanged acid of M.P. 280°C was again isolated.

(4) A similar experiment as in (3) with ethyl-alcoholic ammonia gave the same negative results.

SUMMARY.

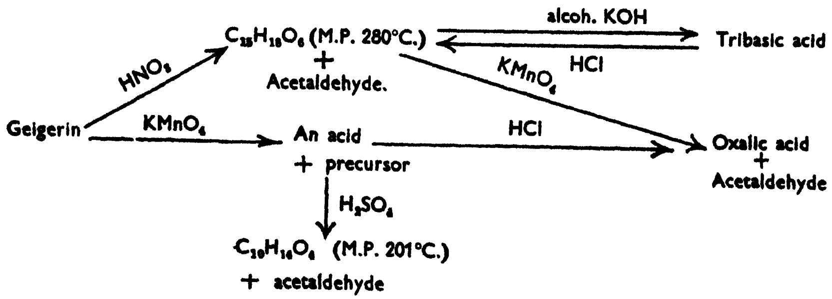
1. Based on the results already obtained on the constitution of the bitter principle Geigerin (*Geigeria aspera*, Harv.) a preliminary discussion as to its structure is put forward in this paper. A more detailed discussion as to the constitutions of Geigerin and the active principle Vermeeric acid is reserved for a later date.

2. Nitric acid oxidation of Geigerin yields an acid $C_{15}H_{18}O_6 \cdot H_2O$ together with acetaldehyde.

3. The $C_{15}H_{18}O_6 \cdot H_2O$ (M.P. 280°C) acid is a mono-carboxylic-dilactonic acid, thus the functions of all six oxygen atoms are known. On saponification with alcoholic potassium hydroxide a tribasic acid results which may again be relactonized to the dilactonic-monobasic acid. It has $\left[\alpha\right]_D^{25} = +181 \cdot 85°$, is very stable but can be oxidised with alkaline potassium permanganate to oxalic acid.

4. Oxalic acid is also a direct oxidation product of Geigerin with potassium permanganate. It is interesting to note that quite a number of bitter principles e.g. Alanto-lactone, Artemisin and Pikroglobularin (Abderhalden, 1936) also yield oxalic acid when oxidised with potassium permanganate. A nitric acid oxidation of Podophyllotoxin (Abderhalden, 1936, p. 162) also led to oxalic acid.

5. These constitutional results may best be summarised as follows:



6. Basing on the information so far obtained the view is expressed, that the fourth oxygen atom of Geigerin may be constituted in a hydroxyl-group (instead of ether-bound or cyclic) and that Geigerin might belong to the sesquiterpene class of substances.

Acknowledgement.

In conclusion I wish to thank Dr. Rimington of this department for his kind interest in this research.

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The Detection of Strychnine in Carcasses and Corpses.

By DOUW G. STEYN, Section of Pharmacology and Toxicology, Onderstepoort.

[Continued from *Onderstepoort Jour. Vet. Sc. and Anim. Indust.*, Vol. 5, No. 1, 1935, pp. 139–174.]

In a previous paper (Steyn, 1935) the tests employed in the detection of strychnine, the length of the period after death during which strychnine is detectable in corpses and carcasses, and recommendations as to the most reliable method of diagnosing strychnine poisoning were fully discussed. This discussion was based on experiments conducted by the author upon twenty-four dogs killed with strychnine and five control dogs which were shot and buried on 7th September, 1933. The remains of these twenty-nine dogs were again exhumed on the 1st and 2nd November, 1937, that is, approximately four years and two months after they were killed and buried. The bones were found to be dry, devoid of fat, brown in colour, and most of them were brittle. The method of extracting the bones was the same as that employed with the organs collected on previous exhumations (Steyn, 1935), namely the Stas-Otto process. The acidified extracts of the bones varied from light yellow to dark reddish brown in colour. No emulsions formed during the process of shaking out with chloroform. As recommended by the author in his previous paper (Steyn, 1935) the taste, potassium bichromate-sulphuric acid (Otto test), and biological tests were applied to the evaporated chloroform residues.

The results of the tests conducted with the bones of the twenty-four dogs killed with strychnine are incorporated in the following table (Table I):—

TABLE I.

Carcasses of Dogs Killed with Strychnine on 7th September, 1939.

Dog No.	Weight of dry bones exhumed and extracted.	Tests for Strychnine.
1247	150 gm.	Otto's test—yellowish-green discolouration which changed to brown Taste—tasteless. Injected mouse developed slight transient apathy and paresis.
1253	260 gm.	Otto's test—slight violet discolouration as in strychnine. Taste—tasteless. Injected mouse developed no symptoms of poisoning.
1309	200 gm.	Otto's test—yellowish-green discolouration. Taste—slightly bitter, not persistent. Injected mouse developed no symptoms of poisoning.
1310	150 gm.	Otto's test—yellowish-green discolouration which changed to dark brown. Taste—tasteless. Injected mouse developed transient symptoms of paresis in hind-quarters.
1311	400 gm.	Otto's test—slight, but definite, violet colour as in strychnine. Taste—slight transient bitter taste. Injected mouse developed symptoms of paresis within 20 minutes after injection and was paralysed within a further 30 minutes. Death occurred 1½ hours after injection.
1312	400 gm.	Otto's test—slight indefinite violet colour which changed to reddish-brown. Taste—not bitter. Injected mouse developed no symptoms of poisoning.
1313	220 gm.	Otto's test—light green colour reaction which changed to light brown. Taste—tasteless. Injected mouse developed dyspnoea, paresis and progressive paralysis within 3 minutes after injection. Died within 1 hour after injection.
1314	400 gm.	Otto's test—dark-green colour. Taste—slightly bitter taste. Injected mouse developed clonic spasms of the muscles of the fore-quarters which was followed by paresis and complete paralysis. Death within 50 minutes.
1317	400 gm.	Otto's test—slight transient violet discolouration as in strychnine. Taste—faint, but definitely bitter. Injected mouse developed dyspnoea and progressive paralysis within 2 minutes after injection. Complete paralysis set in 5 minutes after injection. Died within 45 minutes.
1318	400 gm.	Otto's test—light green discolouration which changed to dark brown Taste—faint bitter taste. Injected mouse developed paresis and general paralysis within 10 minutes after injection. Death occurred within 65 minutes after injection.

TABLE I (*continued*).

Dog No.	Weight of dry bones exhumed and extracted.	Tests for Strychnine.
1319	550 gm.	Otto's test—light green colour. Taste—very slight bitter taste which persists for a while. Injected mouse developed symptoms of general paralysis, and died within 1 hour.
1320	400 gm.	Otto's test—light green discolouration. Taste—bitter taste. Injected mouse developed transient symptoms of paresis in the hindquarters.
1321	350 gm.	Otto's test—dark green discolouration which changed to brown. Taste—faint bitter taste. Injected mouse reacted as in No. 1320.
1322	550 gm.	Otto's test—dark green discolouration which changed to brown. Taste—tasteless. Injected mouse reacted as in No. 1320.
1323	600 gm.	Otto's test—dark-green colour. Taste—slight bitter taste. Injected mouse developed no symptoms of poisoning.
1324	400 gm.	Otto's test—brick-red colour reaction which changed to light green. Taste—intense, persistent bitter taste. Injected mouse remained normal for about 3 minutes; subsequently it showed pronounced hypersensitivity with symptoms somewhat resembling those of strychnine.
1325	700 gm.	Otto's test—persistent yellow colour. Taste—bitter taste which persists for about 1 minute. *N.B.*—Extracted with amyl alcohol by mistake. Injected mouse developed symptoms of paresis within 10 minutes and then continuous, quick clonic strychninelike spasms of the whole body. It died within 1½ hours after injection.
1328	320 gm.	Otto's test—dark-red colour which changed to dark-brown. Taste—slight bitter taste. Injected mouse developed no symptoms of poisoning.
1329	410 gm.	Otto's test—yellow colour reaction. Taste—intense persistent bitter taste. Injected mouse developed dyspnoea and progressive paralysis within 2 minutes after injection and died 20 minutes after injection.
1330	550 gm.	Otto's test—dark green discolouration. Taste—tasteless. Injected mouse became hypersensitive and developed transient paresis of the hindquarters within 10 minutes after injection.
1331	500 gm.	Otto's test—yellowish-green discolouration which passed into brown. Taste—intense persistent bitter taste. Injected mouse developed slight paresis and recovered 3 hours after injection.

TABLE I (*continued*).

Dog No.	Weight of dry bones exhumed and extracted.	Tests for Strychnine.
1332	550 gm.	Otto's test—dark green discolouration. Taste—tasteless. Injected mouse became apathetic and developed transient paresis of the hindquarters.
1333	500 gm.	Otto's test—yellowish-green discolouration which changed to brown. Taste—not bitter. Injected mouse developed progressive paresis and paralysis within 3 minutes and died after 1 hour.
1334	400 gm.	Otto's test—light green discolouration which changed to brown. Taste—tasteless. Injected mouse developed no symptoms of poisoning.

From the above table it is evident that no strychnine is detectable in the remains of any of the twenty-four dogs killed with strychnine and exhumed four years and two months after burial. In four cases (dogs 1253, 1311, 1317 and 1312) the chloroform residue showed a slight violet discolouration with the potassium bichromate-sulphuric acid test. This colour reaction was, however, most probably not due to the presence of strychnine, as in each case the biological test was negative for strychnine. With the exception of six mice (1253, 1309, 1312, 1323, 1328 and 1334) all of them developed symptoms of paresis and general paralysis, which is probably due to the presence of ptomaines in the injected solutions. In a number of cases the chloroform residue contained a fair amount of an amorphous white powder (ptomaines?). The author has again confirmed the results of previous experiments in regard to the relative sensitivity of the potassium bichromate-sulphuric acid test and the biological test, in that he was able to detect as little as 0·004 mg. of strychnine* by means of intraperitoneal injections into white mice. The chloroform residues were taken up in 1·0 c.c. of physiological saline solution slightly acidified with hydrochloric acid and injected intraperitoneally into 14 day old white mice weighing 5 to 6 gm. With the potassium bichromate-sulphuric acid test no violet colour was detectable with quantities of strychnine smaller than 0·007 to 0·01 mg. It is therefore evident that the biological test, in which 14 day old white mice are employed is approximately twice as sensitive as the chemical test (Otto's test).

* In the previous paper (Steyn, 1935) page 172, paragraph (2) "strychnine sulphate" should read "strychnine."

Control Dogs.

The results of experiments conducted upon the five control dogs are quoted in the following table:—

TABLE II.

Carcasses of Control Dogs Shot on 7th September, 1933.

Dog No.	Weight of dry bones exhumed and extracted.	Effects of extracts of the bones injected intraperitoneally into 14 day old white mice (5–6 gm. in weight).
1251	300 gm.	Otto's test—dark green discolouration. Taste—pronounced transient bitter taste. Injected mouse developed no symptoms of poisoning.
1315	500 gm.	Otto's test—yellowish-green discolouration. Taste—slightly bitter. Injected mouse developed general paresis within 5 minutes after injection and was paralysed within a further 5 minutes. When prostrate the mouse suddenly developed repeated attacks of clonic spasms of the whole body musculature. This lasted until death which occurred 3¼ hours after injection.
1316	500 gm.	Otto's test—greenish discolouration, which passed into brown. Taste—tasteless. Injected mouse developed slight transient apathy and paresis.
1326	600 gm.	Otto's test—dark-green discolouration. Taste—fairly pronounced bitter taste. Injected mouse developed slight paresis of all the muscles and recovered within ½ hour
1327	410 gm.	Otto's test—slight violet discolouration which changed to light brick-red. Taste—not bitter. Injected mouse became paretic 3 minutes after injection, but recovered after 1½ hours.

It is of great interest and extreme importance to note that the evaporated chloroform residue prepared from the bones of dog 1327 yielded a colour reaction with potassium bichromate and sulphuric acid which bore a marked resemblance to that seen in positive tests for strychnine. The result of the more sensitive biological test, however, excluded the possibility of strychnine being present. The slight positive colour test for strychnine was therefore probably due to the presence of ptomaine(s).

Also in the case of the control dogs the symptoms of paresis and paralysis, induced in the injected mice by the evaporated chloroform residues were probably due to the presence of ptomaines.

Discussion and Conclusions.

In the course of experiments conducted upon twenty-four dogs killed with strychnine and upon five control dogs, and of analysis of specimens of animal organs submitted in cases of suspected

strychnine poisoning the author had occasion to analyse hundreds of specimens for the presence of strychnine. From the results of these investigations the following conclusions can be drawn:—

(1) Serious mistakes in the detection of strychnine can be made if only colour tests are applied to the material to be tested. It is absolutely essential that three different tests, namely the taste test, a colour test (potassium bichromate-sulphuric acid test) and the biological test be applied. The biological test at the same time allows of a fairly accurate quantitative determination of strychnine. It has repeatedly been experienced by the author that extracts of animal organs submitted for analysis have yielded a fairly distinct positive Otto test for strychnine, whilst it was definitely proved by means of biological tests upon white mice and by the taste test that no strychnine was present in the extracts. From Tables I and II it is evident that extracts prepared from the bone of four dogs (1253, 1311, 1317 and 1312) killed with strychnine and from the bones of one control dog (1327) yielded colour reactions with the Otto test which could easily be mistaken for those of strychnine. *Our one and only safeguard is the biological test.* The author therefore cannot but agree with Gadamer (1924) and Wrede (1937) who state that the colour tests for strychnine, when only small quantities of material are available, cannot be exclusively relied upon but should be confirmed by the biological test. Van Itallie and Bylsma (1930) refer to repeated statements made in the literature *that ptomaines in decomposing carcasses and corpses may yield colour reactions, especially with the Otto test, which very closely resemble those seen in strychnine.* Van Itallie and Bylsma further state that no ptomaines are known which yield both a positive chemical and biological test. Extensive experience gained by the author in the course of the last twelve years definitely confirms the above statements made by various authors.

In order to conduct both the Otto test (potassium bichromate-sulphuric acid test) and the biological test, using 14 day old white mice, it is possible to detect quantities of strychnine as small as 0·011 mg.

In the extraction of strychnine from human and animal organs, Stewart, Chatterji and Smith (1937) recommend that the protein- and fat-containing material be precipitated by grinding the material to be tested with an equal volume of a 10 per cent. trichloracetic acid solution. The filtrate is then shaken with káolin which absorbs the strychnine. It has, however, as yet to be established whether it would be possible to isolate quantities of strychnine as small as can be done with the Stas-Otto process carefully executed. There is a possibility of a certain amount of the strychnine present in the specimen being removed with the proteins and fats during the process of precipitation by means of trichloracetic acid and this would certainly be undesirable in cases where only minimal amounts of strychnine are present in the specimens to be analysed.

Wrede (1937) states that he found his method of determining and weighing strychnine as the aurochlorate more satisfactory than the Stas-Otto process. He was able to determine quantities of

strychnine as small as 0·1 mg. From facts stated above it is evident that the method suggested by the author, namely, the Otto colour test and the biological test, allows of the determination of much smaller quantities of strychnine ($\pm$0·011 mg.). In the course of routine analysis of specimens of animal organs submitted for the strychnine test the entire specimens were frequently found to contain less than 0·1 mg. of strychnine.

(2) It appears that the view that strychnine is still detectable in carcasses and corpses for periods up to twelve years and longer, after death, is fallacious. In twenty-four dogs killed with approximately 5 m.l.d. of strychnine sulphate it was impossible to detect strychnine in any one of them four years and two months after death and burial. No strychnine was detectable in some of these carcasses within eighteen weeks after death, and in four out of eight carcasses exhumed eleven months after death it was impossible to detect strychnine.

It therefore appears that strychnine, like so many other organic poisons, disappears from carcasses and corpses in due course of time, although it appears to resist destruction to a fair extent during the processes of decomposition.

Summary.

1. A description is given of the results of experiments conducted upon twenty-nine dogs in order to ascertain (*a*) for what period after death strychnine is still detectable in carcasses and corpses, and (*b*) what tests are essential in the testing of materials for the presence of strychnine.

2. *It has been definitely established that it is absolutely essential to apply the taste test, colour test (Otto's test) and biological test to materials (purified extracts) to be tested for the presence of strychnine, especially when only minimal quantities of strychnine are present in such materials.* This statement is made with special reference to the detection of strychnine in carcasses and corpses, as it has been established that there is a possibility of decomposition products (ptomaines) being present which yield colour reactions similar to those seen with strychnine.

It is obvious that in forensic medicine it is of the utmost importance to bring absolute proof of the presence or absence of strychnine in carcasses and corpses, hence the necessity for applying all three the above-mentioned tests.

3. Strychnine, like so many other organic poisons, disappears from carcasses and corpses in the course of time. The view so generally held that strychnine is still detectable in carcasses and corpses for periods up to twelve years, or longer, after death appears to be fallacious.

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Section VI.

Pathology.

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Quantitative Studies upon Porphyrin Excretion in Bovine Congenital Porphyrinuria (Pink Tooth) No. I.

By CLAUDE RIMINGTON and G. C. S. ROETS, Section of Toxicological Chemistry; and P. J. J. FOURIE, Section of Hygiene, Onderstepoort.

THE occurrence of congenital porphyrinuria in a bovine herd has been recently reported (Fourie 1936, Fourie and Rimington 1937) and a study made of the types of porphyrin present in these cases Rimington 1936 and 1937, Rimington and Roets 1937).

Since the literature reveals little accurate information as to the quantities of porphyrins excreted in congenital porphyrinuria, studies of these bovine cases have been undertaken with a view to ascertaining whether or not fluctuations occurred in the quantities of copro- and uroporphyrin eliminated with the urine and faeces and if any correlation was to be observed between such data as urine volume and porphyrin output. Not only was it hoped that the results would contribute materially to the elucidation of the disease, considered as a derangement of endogenous pigment metabolism, but we were also anxious to throw further light upon the uneven distribution of porphyrin in the bones of affected animals. The case previously slaughtered for examination showed the bone structure, in transverse section, to be stained unevenly in rings of darker and lighter colour (compare Fink and Hoerburger 1935).

HISTORICAL.

It must be stated at the outset that the distinction usually drawn between congenital (including the so-called chronic cases) and acute idiopathic porphyrinuria is by no means a wholly satisfactory one. Thus, in the former type, excretion of porphyrin is generally regarded as being practically continuous from birth (congenital) or sometime after (chronic) whilst in the acute form of the disease porphyrin excretion is paroxysmal. Similarly the pigments of the congenital type belong mainly to the I series (but compare Fischer and Hofmann 1937, Rimington 1936, 1937) whilst those of acute porphyrinuria are preponderately series III derivatives (Waldenström 1935, Mertens 1936, Dobiner 1936). Transition forms between the "chronic" and "acute" types of the disease are occasionally to be encountered (van den Bergh, Regniers and Muller 1928, van den Berg and Grotepass 1937 and Dobiner 1936).

Prior to Hans Fischer's investigation of the "Petry" case and isolation of copro- and uroporphyrin, workers in this field were wont to refer to the mixture of pigments in urine or faeces as "haematoporphyrin". The methods they used for quantitative determination were crude (see Garrod 1923, Günther 1911, 1922) and no very great reliance can be placed upon their figures. Nevertheless Garrod mentions cases in which pigment excretion was intermittent and thus like that of Arzt and Hausmann (1920) in which the colour of the urine varied appreciably at different periods and this has also been the experience of other workers. Fischer estimated that Petry excreted about 0·4 gm. total porphyrin per day.

Quantitative investigations using refined methods of analysis have recently been carried out upon copro-porphyrin III excretion in lead poisoning (Mertens 1937) in which case it was found that there was a definite tendency for urinary porphyrin and urine volume to run a parallel course. From Dobiner's recent work (1937a; 1937b) upon porphyrin excretion in various pathological states it is difficult to tell whether any such regularity occurred. He states that the daily copro- and uroporphyrin excretion varied considerably but calculating daily averages over 3 to 10 day periods of observations, the figures for these periods were comparable.

Methods.

The animals were kept in metabolism stables provided with a channel for collecting the urine in a glass receptacle and in order to minimise possible contamination with faecal material, assistants were employed day and night whose duty it was to shovel up the faeces directly they were passed into weighed tins. The animals were maintained upon the usual laboratory ration and watered from a bucket twice daily. Collection extended over 5 to 6 day periods and each daily batch of material was worked up immediately.

Urines.—The volume was measured and an aliquot of 150 to 300 c.c. (according to porphyrin content) acidified with acetic acid to a final concentration of 5 per cent. and the mixture then extracted with ether for 24 hours in a Kutscher-Steudel continuous extraction apparatus. All ether-soluble porphyrin was in this way removed and troublesome emulsions avoided. The ether extract was washed in a separatory funnel with water containing potassium acetate until the bulk of the acetic acid had been removed. The water washings usually contained urobilin or a similar pigment. The total porphyrin was now transferred, by shaking, with 5 per cent. hydrochloric acid and this solution made up to a convenient volume. An aliquot usually 5 c.c. was then placed in a test tube and diluted with water until the intensity of the absorption band at 550 matched that of a standard solution, similarly observed by means of a pocket spectroscope, and containing 1 mg. coproporphyrin in 100 c.c. of 5 per cent. hydrochloric acid. With practice small differences between test and standard solutions could easily be detected but we put the error of the whole determination on the conservative side as ± 10 per cent. Colorimetric comparison of the acid solutions would have been possible and gave in check experiments nearly identical results, but

we preferred to base our determinations upon a specific character such as the intensity of an absorption band rather than on anything so unspecific as colour.

Uroporphyrin was determined in the ether extracted urine by an analogous process after conversion into the more stable copper complex. In preliminary experiments an endeavour was made to purify and concentrate the pigment by filtering it through adsorption columns of alumina and then eluting with diluted alkali but this procedure proved both troublesome and time-consuming. Finally, direct determinations were made as follows: The ether-extracted urine was transferred to a 200 or 500 c.c. graduated flask and made up to the neck with a 5 per cent. sodium hydroxide, the container and spiral of the Kutscher-Steudel apparatus being rinsed out with the same solution. The final reaction of the mixture was alkaline and a copious micro crystalline precipitate separated leaving a clear yellow-brown supernatant liquid. An aliquot of 10 c.c. of this was transferred to a 50 c.c. flask, two drops of saturated aqueous copper acetate solution added and the mixture digested on a warm plate for five minutes using a lightly fitting glass stopper as a hang-in condensor to prevent loss of liquid by evaporation. The reaction mixture was then filtered, a 5 c.c. aliquot placed in a small test tube and the intensity of the 569·5 band of the uroporphyrin copper complex spectrum compared with that of a standard prepared from pure uroporphyrin and copper acetate. A series of tubes was kept containing dilutions of the standard equivalent to 1, 1·2, 1·4, 1·6, 1·8 and 2·0 mgm. pigment per 100 c.c. and the concentration in the unknown estimated to the nearest tenth. Thus an unknown stronger than the 1·6 but weaker than the 1·8 standard was said to contain 1·7 mgm. per 100 c.c. Admittedly the error of determination is here again about ± 10 per cent. but the method was rapid. No other method is known permitting of the quantitative determination of uroporphyrin in such complex mixtures with any higher degree of accuracy. Separation from the accompanying brown and yellow pigments of the urine requires an elaborate technique and inevitably entails serious losses. When determination is based upon the intensity of an absorption band, simultaneous presence of other non-specific absorbing pigments has but a negligible effect.

Faeces.—The quantity of coproporphyrin excreted per rectum was determined as follows: The entire faecal mass passed during 24 hours was mixed as intimately as possible and after weighing an aliquot of 100 gms. was subjected to the usual extraction technique with acetic acid and ether. The ether-soluble porphyrin was finally passed into 5 per cent. hydrochloric acid, this solution shaken with chloroform if necessary and the intensity of the 550 absorption band matched against the standard as previously described. Duplicate determinations upon both faeces and urine yielded consistent results.

Results.

A summary of the numerical results is presented in tables and also graphically in the diagrams Figs. 1 and 2.

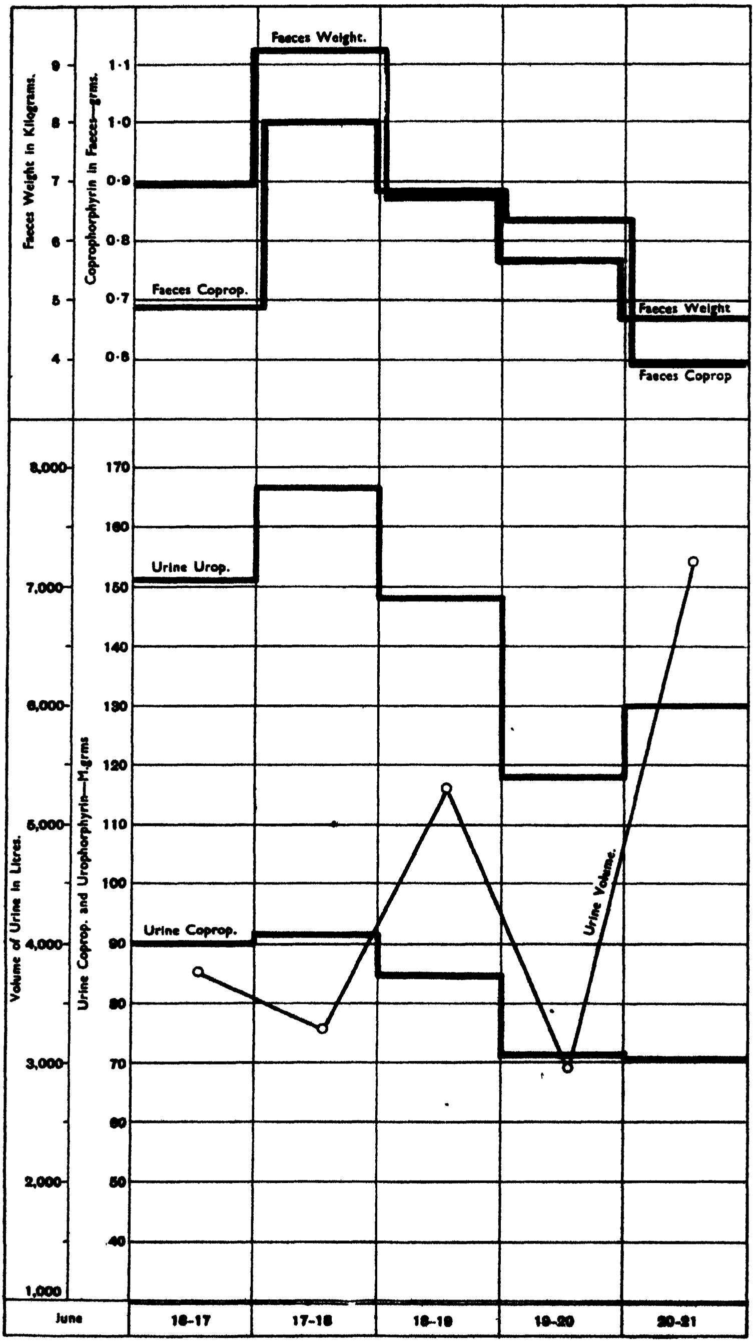
Faeces Weight in Kilograms.
Coprophorphyrin in Faeces—grms.
Faeces Weight.
Faeces Coprop.
Faeces Weight
Faeces Coprop
Volume of Urine in Litres.
Urine Coprop. and Urophorphyrin—M.grms
Urine Urop.
Urine Coprop.
Urine Volume.
June
16-17
17-18
18-19
19-20
20-21

Fig. I.

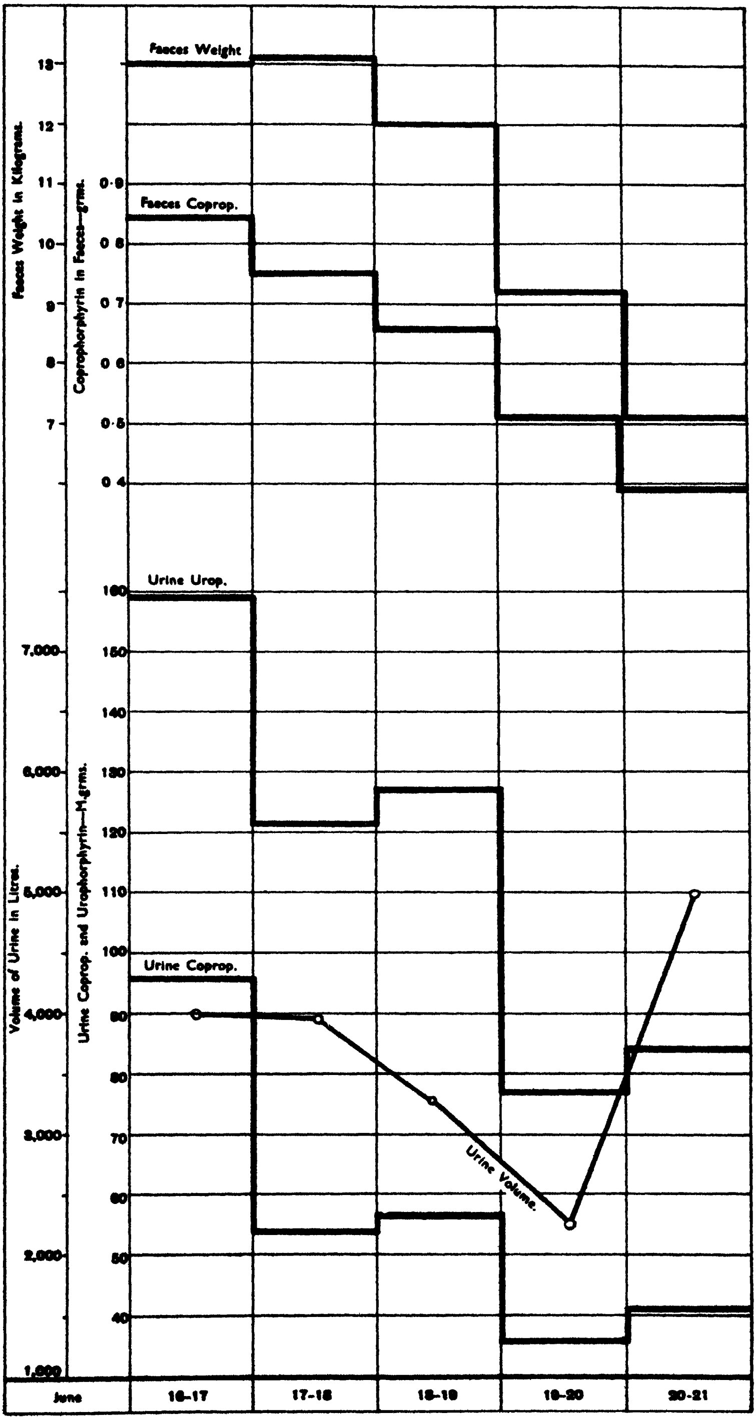
Faeces Weight
Faeces Coprop.
Urine Urop.
Urine Coprop.
Urine Volume.
Faeces Weight in Kilograms.
Coprophorphyrin in Faeces—grms.
Volume of Urine in Litres.
Urine Coprop. and Urophorphyrin—M.grms.
June
16-17
17-18
18-19
19-20
20-21

Fig. 11.

It is clear from an examination of this data that the excretion of porphyrin by any one animal varies greatly from day to day. A similar circumstance has been found in the case of normal dogs by Dobiner (1937c). The period of three consecutive days utilised for the first observation (February 10-12) is clearly too short to allow of any calculation of an average daily excretion. When however the periods March 1-6 and June 16-20 are compared it is sure that both bovine 7017 and 7018 excreted on the daily average considerably more porphyrin during the later period. That this was not due to any error in technique was proved by making up new standards and repeating the observations. We can only suggest that possibly season or climate influenced the porphyrin excretion for it may be noticed that the weather during March was still hot whilst June was cold with temperatures well below freezing point at night. Whether or not this explanation is correct can only be ascertained by an extended series of observations throughout the year.

In assessing the significance of the data presented, the following points should be borne in mind:—

1. Both coproporphyrin and uroporphyrin are of endogenous origin.
2. Porphyrin excretion takes place via the bile and urine thus the relative quantities appearing in the faeces and urine will depend largely upon the balance of factors effecting biliary and renal secretion.
3. Uroporphyrin is also retained in the body being absorbed by growing bony tissue.
4. The exact generic relation between coproporphyrin and uroporphyrin is not known. There is a possibility that uroporphyrin may arise in part from coproporphyrin in the kidney.

An examination of the data does not reveal any precise quantitative relationship between the two types of porphyrin. In seeking correlations between urine volume and porphyrin excretion, the period June 16-20 is the most satisfactory since during this period very wide fluctuations occurred both in urine volume and faeces weight. It would appear that:—

1. There is no correlation between either urinary copro- or uroporphyrin and urine volume. In fact the tendency is towards a constancy of porphyrin output irrespective of the total quantity of liquid passed. Such behaviour is in opposition to that seen in lead poisoning where the quantity of coproporphyrin III voided in the urine rises and falls with the urine volume (Mertens 1937).
2. Coproporphyrin and uroporphyrin seem to run a parallel course in the urine, the quantities of these pigments rising and falling in sympathy.
3. The output of coproporphyrin in the faeces is related to faeces weight. This does not indicate any exogenous origin but is rather an expression of the circumstance that more bile passes into the alimentary tract and is voided when a large bulk of food material is passing through.

4 The total quantity of porphyrin excreted daily is subject to wide variations being chiefly influenced by the magnitude of the faeces component, but remains approximately constant when the daily average over a five or six day period is considered. The highest excretion recorded was 1·27 gm. porphyrin during 24 hours.

5. Further work must be undertaken to ascertain whether or not there is any seasonal rhythm of porphyrin excretion.

Identification of the Porphyrin Excreted.

From the urine of these cases uroporphyrin ester of M.P. 275-7° was isolated and resolved into uroporphyrin I (M.P. 292-3°) and smaller quantities of uroporphyrin III (M.P. 260°). (Rimington 1936.)

In the case of coproporphyrin fraction, this has recently been separated by Rimington and Roets (1937) into coproporphyrin I and coproporphyrin III. It was highly desirable, however, that the relative proportions of these two isomers should be known and accordingly the following experiment was performed with this object in view:

2·8 litres of freshly passed urine (bovine 7017) was acidified by acetic acid and extracted in a Kutscher-Steudel apparatus with ether until no more porphyrin was removed by the solvent. The ether solution was then worked up in the usual way and the total quantity of coproporphyrin measured by comparison of the 5 per cent. acid solution with the standard coproporphyrin.

Volume of solution 500 c.c.

5 c.c. aliquot was diluted to 29·5 c.c. to match standard of 1 mgm./100 c.c. therefore 29·5 mgm. porphyrin in all.

The pigment was transferred to ether, esterified and the total ester obtained by evaporating the chloroform solution to dryness. On stirring with cold methyl alcohol, a portion dissolved. This was coproporphyrin III and its quantity was ascertained by matching against the standard. The quantity of coproporphyrin I remaining was similarly determined.

Coproporphyrin	III	1·06 mgm.
,,	I	29·11 mgm.

In order to be sure that No. I series isomer accompanied the III, the methyl alcoholic solution of the latter was evaporated to dryness and again treated with cold methyl alcohol when it dissolved completely. The M.P. of the crystalline material was 120-130°. The purified coproporphyrin I fraction had M.P. 245-7°. From the above figures it is seen that in the urine of this animal the

$$\text{ratio} \quad \frac{\text{coproporphyrin I}}{\text{coproporphyrin III}} = \frac{27\cdot 6}{1}$$

Similar experiments are in progress to determine the ratio in the remaining cases and also the ratio exhibited by the faeces porphyrins.

Repeated recrystallization of a coproporphyrin I specimen isolated from faeces had M.P. 250°.

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APPENDIX.

BOVINE No. 7017.

Date.	Faeces. Weight Kgm.	Faeces. Coprop. mgm.	Urine. Volume litres.	Urine. Coprop. mgm.	Urine. Urop. mgm.	Total porphyrin gm.
February—						
10	9·90	545·0	2·93	33·2	64·5	0·6427
11	8·70	870·0	2·89	62·6	74·2	1·0068
12	9·00	675·0	3·38	49·1	128·9	8·8530
March—						
1	6·20	418·6	2·82	74·1	64·0	0·5567
2	10·90	626·9	2·10	57·8	59·8	0·7444
3	11·60	664·1	3·71	71·9	64·0	0·8000
4	10·10	636·3	3·73	65·3	67·1	0·7687
5	12·50	551·4	4·50	64·7	87·7	0·7038
6	9·30	_627·8_	2·76	_60·4_	_66·2_	0·7544
Mean	—	587·5	—	65·7	68·1	0·7213
June—						
16	6·90	690·0	3·77	90·4	150·6	0·9310
17	9·2	1,012·0	3·28	91·8	166·2	1·2700
18	6·8	884·0	5·30	84·8	155·5	1·1243
19	5·7	627·0	2·95	70·8	118·0	0·8158
20	4·7	_564·0_	7·20	_70·1_	_131·0_	_0·7651_
Mean	—	755·4	—	81·6	144·3	0·9812

BOVINE No. 7018.

Date.	Faeces. Weight Kgm.	Faeces. Coprop. mgm.	Urine. Volume litres.	Urine. Urop. mgm.	Urine. Coprop. mgm.	Total porphyrin gm.
February—						
10	12·40	477·0	3·17	56·5	74·3	0·6078
11	12·90	537·0	2·05	39·7	146·5	0·7232
12	11·50	409·0	1·88	20·9	73·5	0·5034
March—						
1	13·90	469·2	2·12	23·6	48·1	0·5409
2	10·50	420·0	1·44	23·0	46·1	0·4891
3	13·10	467·9	2·34	21·1	48·4	0·5374
4	13·00	435·2	3·19	23·4	45·7	0·5043
5	10·5	525·0	6·90	50·6	42·1	0·6177
6	8·7	_398·7_	4·90	_17·2_	_71·9_	0·4878
Mean	—	452·7	—	26·5	50·4	0·5295
June—						
16	12·95	841·8	4·00	96·0	159·4	1·0972
17	13·10	786·0	3·95	54·1	121·7	0·9618
18	12·00	660·0	3·29	56·4	126·9	0·8433
19	9·20	510·6	2·25	35·9	77·0*	0·6235
20	7·10	_390·5_	4·95	_41·1_	_84·0_*	_0·5156_
Mean	—	637·8	—	56·7	113·8	0·8083

* Possibly some loss by overflowing.

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A Further Case of Congenital Porphyrinuria (Pink Tooth) in a Living Grade Friesland Cow in South Africa. (Cedara Case).

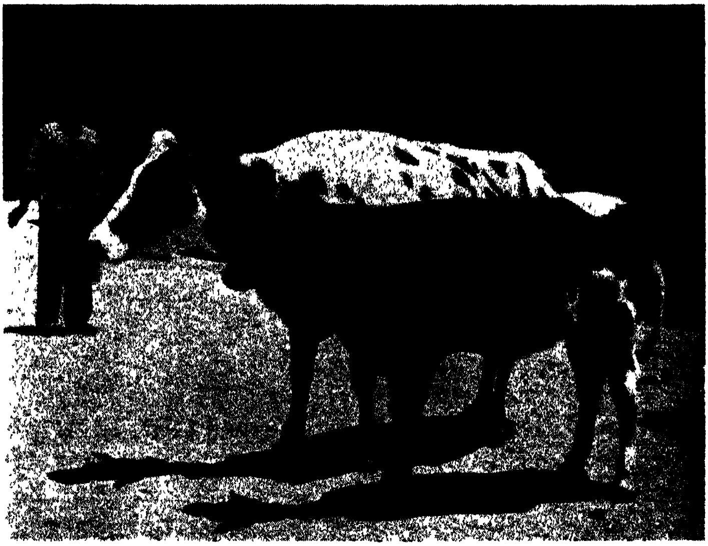
By P. J. J. FOURIE, Section of Hygiene, and C. R. RIMINGTON, Section of Toxicological Chemistry, Onderstepoort.

DETAILS concerning the occurrence, symptomatology, pathology and haematology of the first and only known living world cases of congenital porphyrinuria in bovines, were recorded by Fourie (1936).

These cases were discussed by Rimington (1936) independently from the chemical point of view. These cases all occurred in a grade short horn herd soon after the introduction of a certain bull as sire. Fairly extensive enquiries were at that time made but no definite evidence was obtained as to the presence of other clinical cases in South Africa. However, one of our Colleagues, Dr. I. P. Marais, whilst examining the cattle of the Cedara School of Agriculture, Natal, for mastitis, found one cow, showing extensive skin lesions and having at the same time discoloured teeth. The urine was not available for examination, but otherwise the clinical picture was that of congenital porphyrinuria. This cow was taken over by the Division of Veterinary Services and is the subject of this communication. She will for the sake of convenience be referred to as the Cedara case.

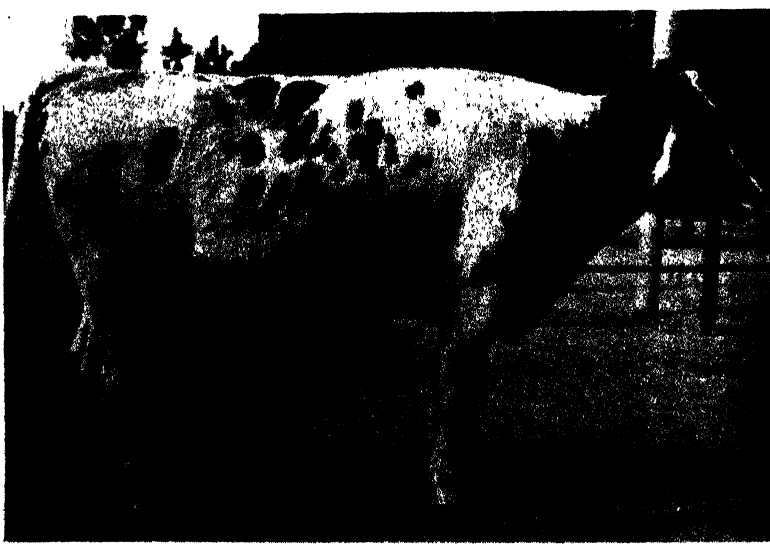
History.—She is a Grade Friesland cow (177, C. Ria 4th, Onderstepoort No. 7354) born at Cedara 20.12.1930. Her dam and granddam (the latter being in 1937 more or less 17 years old, born 25.8.1920) are both alive. Her sire C. Joe is dead. As a calf she had three sores on her body. As far as can be made out these never healed. She was a shy breeder. Her first date of service was 24.3.33. Her first pregnancy resulted in an abortion on the 18th March 1936. Subsequently she was repeatedly served, but eventually held to a service, as a result of which she produced, here at Onderstepoort, a beautiful, normal, high grade Friesland, heifer calf on the 18.5.1937. (Figs. 1 and 2).

Figure 1.



Cedara Case: Cow 7354 with her calf No. 7393.

Figure 2.



Cedara case: early skin lesions between black spots towards the top of the back. Lesions do not show up too well in photograph.

This is the first living calf known to have been delivered by a bovine affected with congenital porphyrinuria.

Ancestry.—The hereditary nature of porphyrinuria will not be discussed in this paper. The evidence that the condition is hereditarily transmitted as a recessive character will be presented by one of us at a later date. It will nevertheless be of interest to submit here relevant portions of the ancestry of this Cedara case.

Ancestry of the Cedara case: Cow 177. *Cedara Ria 4th.*

Angel:

Ria 136 (*granddam of Cedara case*) *by Elsenburg Rhonda* 985 *V. 3. F. H. B.*

113 *Cedara Ria 2nd* (*dam of Cedara case*) *by Frederick*

177 *Cedara Ria 4th* (*Cedara case*) *by Cedara Joe.*

The cow Angel is of the Friesland type, but she was not registered and nothing is known of her ancestry. She was however a poor doer. At the time this was thought to have been due to liver fluke infestation, but Mr. Johnston, Animal Husbandry Officer of the Stellenbosch-Elsenburg College of Agriculture, who kindly gave us the above details, makes the statement that, " it would appear to be possible that if the cow was destroyed as a bad doer, a rare condition such as porphyrinuria may have been overlooked and the entire trouble attributed to the liver fluke." This of course implies that the cow Angel may have been a clinically affected case, whereas she may equally well have been a carrier of the recessive character (Dr.), in which case she would have been to all intents and purposes a clinically normal animal.

Turning now to the bull Cedara Joe, the sire of the Cedara case, this bull is, as far as is known clinically normal, and must therefore have been a carrier of the recessive character (Dr.). The pedigree of C. Joe is:

Cedara Joe

Cedare Jetske 2nd — Nelsrust Commodore 2nd 1707. Vol. 3.

Ogden Hall Alberta — Nelsrust Beatty 2nd.

Admiral Beatty.

From this it will be seen that C. Joe is descended from the once famous South African Bull Admiral Beatty, which in turn is descended from the more famous sire Jan 3265 F. R. S. of Holland.

Enquiries were made, but no evidence suggestive of any cases of porphyrinuria in the ancestry of the bull C. Joe could be found

However, one should in this connection bear in mind that the chance of recognising even a suspicious case of porphyrinuria in bygone generations is extremely small.

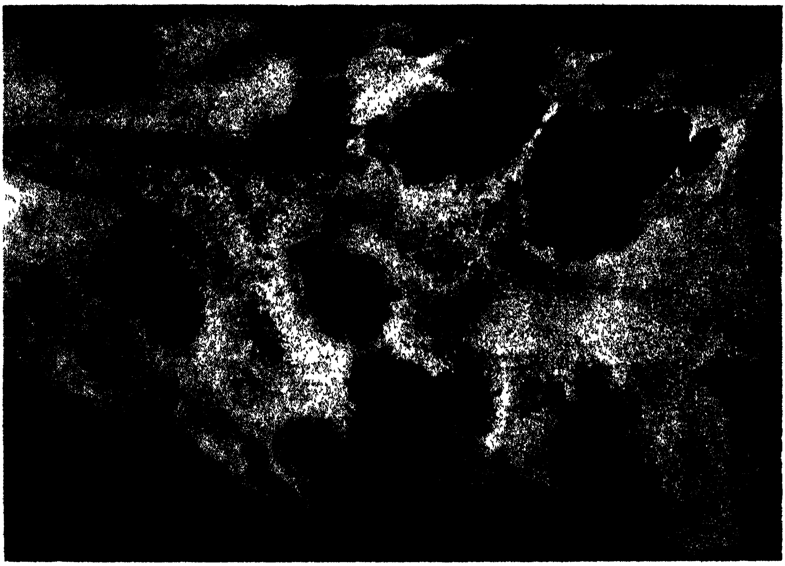
Clinical Features.

General.—The respirations are 36 per minute, the pulse 66 per minute and there are 11 ruminal movements in 5 minutes. Her temperament is docile.

Teeth.—The teeth are reddish brown in colour but definitely not as deeply pigmented as in the shorthorn cases described by Fourie (1936).

Urine.—The urine is of reddish brown colour, having absorption bands at 540·6, 577·6, 497·7, 621·6. On an analysis the urine was found to contain 0·8 mg. of coproporphyrin and 1·4 mg. uroporphyrin per 100 c.c. Porphyrins were also identified in the faeces.

Figure 3.



Cedara case: Old skin lesions on the back.

Conjunctiva.—This is markedly reddened and of a bright pink colour, but there is no noticeable discharge from the eyes. When the animal was stabled to protect her against the harmful rays of the sun the reddening of the conjunctiva gradually diminished, until some time after stabling, the conjunctiva became completely normal in colour again.

Lesions of photosensitazation.—Lesions are present on portions of the unpigmented skin exposed to the direct rays of the sun. The lesions extend along the back, from the withers to the commencement of the tail. See Figs. 3 and 4.

Except for small portions here and there, the lesions are continuous in the skin over the spinous processes. One large lesion extends obliquely from in front and mesial to the external angle of the ilium to about 10 cm. from the tuber ischii. Lesions extend on either side from the top of the back to a distance of 15 cm. down between the last two ribs. These lesions consist of hard, keratinised crusts with horny, fingerlike outgrowths in places. These are old lesions, probably of some years standing, but in some portions of unpigmented skin, young lesions are present. These are in the form of raised wheals, confined to unpigmented parts of the skin, which shows acute reddening, but the black spots on the skin, contiguous to altered unpigmented skin, are completely normal. No lesions are present on the nose or the muzzle.

Figure 4.

Cedara case: Old skin lesions on the back.

Haematology.

A haematological examination of this animal was made on three occasions. Details are given below.

Date.	*R.C.*	*W.C.*	*R.P.*	*L.*	*N.*	*M.*	*E.*	*B.*
24/ 2/37..............	5·3	8·8	—	53	25	4	17	1
20/10/37..............	4·2	6·5	—	54	18	8	19	1
30/11/37..............	4·1	8·2	—	38	40	7	15	0

The red counts are somewhat on the low side, but no well marked morphological changes indicative of anaemia are present. Only occasionally does one see cells showing punctate basophilia.

The eosinophiles are undoubtedly increased in number. In this case bilharzia infection is absent, but unfortunately a liver fluke infestation is present and this may not only be responsible for the low red counts, but also for the eosinophilia. However, haematological observations are being continued in order to see if there is any response to medicinal treatment against liver fluke.

Since writing this article, the cow had an accident as a result of which she sustained a fracture of the external angle of the ilium. The open wound refused to heal and the loose piece of bone was removed and found on analysis (kindly undertaken by Mr. Roets) to contain: Uroporphyrin, the first crop of crystalline methyl ester of which has a melting point of 276°-280°. The spectrum in chloroform shows bands at 626·8, 583·0 571·5, 537·3, 502·7. For comparison the bands of the uroporphyrin ester are quoted, viz. 626·1, 581·4, 570·5, 537·3, 500·8.

SUMMARY.

A further living case of congenital porphyrinuria is described of a cow which gave birth to a clinically normal heifer calf and showing herself discoloured teeth, porphyrin in urine and faeces, and extensive skin lesions of photosensitization.

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Section VII.

Hygiene.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 10, *Number* 2, *April*, 1938.

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A Rapid Phase Test for distinguishing between Carotinoid and Bile Staining of Fat in Carcasses.

By CLAUDE RIMINGTON and P. J. J. FOURIE, Sections of Toxicological Chemistry and Hygiene, Onderstepoort.

In the routine inspection of carcasses a yellow discolouration of the fats is not infrequently observed, but the significance of this discolouration from a meat inspection point of view depends upon whether it is caused by the impregnation of the tissue with bile (icterus) or is due merely to the presence of harmless, or even beneficial pigments of vegetable orrigin (pseudo-icterus). Bile is not normally present in significant quantity in the blood or tissues of the healthy adult pig, sheep or bovine and, therefore, the presence of icterus in any of these species can be said to imply a pathological state.

Green plants contain, associated with their chlorophyll, yellow pigments belonging to the class known as carotinoids and these latter being easily absorbed and fat soluble tend to accumulate in the adipose tissues when an animal is liberally supplied with green food or materials such as yellow maize which also contain appreciable quantities of the pigments.

Although this is the case peculiarities are shown by certain species, the fat of the pig, for example, being usually almost colourless while bovine fat, absolutely free from carotin, is rarely encountered. Furthermore one sometimes finds that in bovines this yellowish discolouration is not confined to fatty tissues but can occasionally be recognised clinically as a yellowish discolouration of the visible mucous membranes.

Carotin discolouration of fat is in no way detrimental, the only support which could be advanced for the popular prejudice against such highly coloured carcasses being the fact that carotin pigmentation of the fat appears to increase in intensity with age.

A PHASE TEST FOR DISTINGUISHING BETWEEN CAROTINOID AND BILE STAINING.

In abattoir practice, however, it becomes necessary to distinguish between bile and carotin staining so that icteric carcasses may be rejected and excluded from sale. For this purpose one or other of the following methods is customarily employed.

1. Visual examination of colour quality. It is said that carotin staining imparts a colour to the fat of a clear yellow as against the more dirty yellow of bile discolouration.
2. The hanging test. A suspected carcass is allowed to hang in the chilled room for 24 hours. Carotin pigmentation is said to decrease and disappear entirely under these conditions, whilst bile pigmentation remains unchanged.
3. A chemical test is performed such as that put forward by Martin or van Manen.

Subjecting to scrutiny each of these methods in turn, it may be said that the first whilst valuable in the hands of an experienced inspector is nevertheless unsatisfactory because it is empirical and lacks precision. Mistakes might conceivably be made in which both diseased carcasses are passed (the import inspector who has not the viscera available is placed at the greatest disadvantage) and, perfectly healthy carcasses condemned because they show a harmless yellow discolouration, mistaken for icterus.

The hanging test is equally unreliable since on the one hand carotin pigmentation may persist for weeks or even months and on the other bile staining has been known to disappear completely overnight (van Manen 1933).

No chemical test so far proposed allows of a rapid result being obtained. Martin (1931) recommends the preparation of an alcoholic extract of the fat to be examined by shaking this with 96 per cent. alcohol for ½ to 2 hours. After filtration a few drops of sulphuric acid are added and the mixture heated when the presence of bile is indicated by the appearance of a greenish colour (due to biliverdin). Van Manen's method is really a modification of Martin's test, practically the same procedure being followed to obtain an alcoholic extract but this is then treated with the Ehrlich diazo reagents when the familiar reddish colour of azo-bilirubin appears.

Both these methods afford information concerning the one pigment only, are time consuming and elaborate and consequently would appear to be only infrequently used.

In addition it may be pointed out that the carotinoids themselves afford brilliant blue colours with strong mineral acids. Thus uncertainty might still be entertained that under the conditions of Martin's test a bluish colour could be due to carotin and not to bilirubin.

It was, therefore, felt that a simple and rapid test to distinguish between bile and carotin staining and which unlike the other tests affords at the same time information concerning the absence or presence of one or the other or of both pigments, would be of considerable value in ordinary abattoir practice. It is claimed that the test to be described below conforms to these requirements.

This test is an extension of that evolved recently (Rimington, 1937) for the examination of sera and is named the phase test since two liquid layers are produced, the upper (ether) one containing any carotinoids present and the lower (sodium hydroxide) retaining the bile pigments. It is performed as follows:—

The fat to be examined should be as free as possible from blood; renal fat is generally very suitable. About 2 gm. is placed in a strong glass test tube with approximately 5 c.c. of a 5 per cent. aqueous solution of sodium hydroxide and the mixture is heated over a flame so that boiling is maintained for about 1 minute. Agitation of the tube aids the disintegration of the fat which takes place during this procedure. The tube is now cooled under the tap until only comfortably warm to the hand (40-50°C). One half to an equal volume of ether is now added and the contents of the tube mixed by careful agitation and then allowed to separate into the two layers. Should persistent emulsion prevent the rapid separation of the phases, this may be overcome by warming gently or by adding a few drops of alcohol.

Since bilirubin forms a water soluble sodium salt it remains in the lower phase which is thereby coloured greenish yellow, the intensity depending upon the quantity of bile which is present. The upper phase will contain any carotinoid pigments, these imparting to the ether a yellow colour. Naturally various combinations are possible, bile alone, carotin alone or mixtures of the two or absence of both and the test will indicate these by a colourless upper and greenish-yellow lower phase, a colourless lower and yellow upper phase, or lastly yellow above and greenish-yellow below or no colour at all. Trial showed that when added to a colourless pork fat, as little as 0·05 c.c. of bile could easily be detected when performing the test.

The chief advantages claimed for the phase test are that it is rapid, requires no special apparatus or chemicals and affords indication of the presence or absence of both carotinoid and bile pigments. By its use decisions based upon reliable criteria could be made with a great saving of time.

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Section VIII.

Sheep and Wool.

Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 10, *Number* 2, *April*, 1938.

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Wool Studies.

III. The Uniformity of a Series of Fibre Thickness Measurements on a Small Sample of Medium Merino Wool.

By A. P. MALAN, Section of Statistics, Onderstepoort; H. B. CARTER, Walter & Eliza Hall Fellow, University of Sydney (Australia); and C. M. VAN WYK, Wool Research Section, Onderstepoort.

INTRODUCTION.

THE fibre diameter of wool, being either directly or indirectly associated with a variety of other characteristics, is required in the majority of problems in wool research. It is essential, therefore, that the procedure of sampling and the technique of preparing wool for diameter measurement should be placed on a fundamentally sound basis. Various characteristic properties of the material however, complicate the establishment of a sound technique of sampling. Wool as such does not readily permit a random selection of individual fibres and any endeavour to select a representative sample of fibres by personal judgment is bound to be biassed. In wool studies sampling is absolutely necessary since the preparation of the whole available material for the measurement of fibre diameter is not only practically impossible in other than very small quantities of wool, but also undesirable because it renders the material useless for further investigations.

The necessity for some adequate system of sampling a quantity of wool is, therefore, a basic consideration, and it is strange, as Wildman (1936) has emphasised, that so little attention has in the past been devoted to this aspect in the assessment of wool characters. The contribution of Fraser Roberts (1930) constitutes about the only comprehensive work in which adequate control of sampling errors was achieved in the course of a series of laboratory determinations

of the average fineness of a sample of raw wool. These investigations involved the use of the weight-length method in determining this character and the system of zoning and sampling used by Roberts therefore has more particular reference to the determination of mean fibre length. In the microscopic measurement of fibre diameter, however, the same principles apply and in practice the method of sampling and of slide preparation is that described by Duerden (1929), and in general use in this laboratory. It consists of zoning the original quantity and selecting at random a number of small staples from each zone. From each such staple a small strand of fibres is drawn without selection and these strands are combined to form the ultimate sample which is prepared for measurement. The preparation consists in cutting this sample into small fragments along the entire length of the fibres or else removing small fragments at intervals along the length. These fragments are thoroughly mixed and a suitable portion removed and mounted on a slide for microscopic reading. The final determination of fibre diameter is therefore made after the original quantity of wool has been reduced in four successive stages each one of which constitutes a process of sampling. These stages are in order, (*a*) the removal of staples from the original zones, (*b*) the taking of small strands from each of these staples, (*c*) the mounting on a slide of a portion of the fibre fragments (*d*) the measurement of a limited number of fibre fragments on the slide. The first two stages comprise the manual process of sampling while the remaining stages involve problems of efficient slide preparation. Both aspects are equally important but sampling methods can only be discussed when it is known that the preparation of the slides is such that the sample will be adequately represented when a suitable number of readings are taken. The representativeness of a series of readings from a slide is dependent upon the thoroughness of mixing of the fragments, and the uniformity of their distribution over the slide.

The microscope method has been followed, because of its many advantages over other methods e.g. the diffraction, weight-length and micrometer caliper methods (van Wyk, 1937). It has also been adopted by the International Wool Conference as the standard method for wool fibre thickness determinations.

Scope of the Present Study.

In the past many disturbing differences between successive slides prepared from the same sample of wool and even between repeated measurements of the same slide were frequently experienced. These differences were often of such a significantly high order that the soundness of diameter measurements by this method was regarded with suspicion. It will be appreciated that these inconsistencies, if beyond reasonable control, would completely nullify the value of fibre diameter determinations by this method and vitally affect many aspects of wool research. Hence it was decided to investigate the whole process of slide preparation and the microscopic determination of fibre diameter.

The observed discrepancies between slides and successive readings of the same slide point to an inadequate mixing of the fibre fragments and to a heterogeneity in the distribution of these fragments over the slide. The present investigation is therefore designed specifically to examine these problems in slide preparation.

A group of ten slides was prepared from each of four mixtures of fibre fragments and each slide was traversed systematically so that twenty-five readings were made in each of ten different areas on the slide. The representativeness of slides, depending on the mixing of the cuttings may be estimated from the variance between consecutive slides while the distribution of fragments over a slide may be estimated from the variance between the ten different localities considered. The observations from these ten localities were recorded in as many columns and thus simultaneously formed twenty-five rows of ten observations each. Hence the 250 measurements from a single slide may be considered as constituting a 10 by 25 Latin square, and the variance analysed accordingly.

The four mixtures of fragments mentioned refer to the four methods of cutting which may be employed in preparing a staple of raw wool for the measurement of fibre diameter. These methods of cutting are referred to as treatments A, B, C and D and are described in the following paragraph.

Two observers each made the complete series of observations using different microscopes. To separate personal differences in the readings as between observers from possible differences due to microscopes (however unlikely this may be) the slides of Treatment A were read a second time. For this purpose groups of five slides from this treatment were allotted at random to each of the two microscopes and these were read in turn by both observers on each instrument.

Description of Technique and Procedure.

The material used in this investigation consisted of a single small staple of medium merino wool (about 66's quality number) and approximately 8·0 cms. ($3\frac{1}{4}$ inches) in length. The quantity taken was such as to represent roughly the amount of wool obtainable from four square centimetres of skin surface on a sheep of medium fleece density. The weight of the sample after thorough scouring with repeated changes of benzol, and conditioning in a humidity chamber at 70°F. and 70 per cent. relative humidity was 0·90 gm. The subsequent handling of the material, until the preparation of the slides was complete, was performed in the humidity room under the constant atmospheric conditions specified.

The staple was divided into ten zones by longitudinal partition so that each zone consisted of wool weighing approximately one tenth of the original weight. These zones were identified by serial numbers 1 to 10.

Treatment A: From each of the 10 zones a small strand of fibres was separated laterally and without selection other than for equality of size. These were combined to form a composite sub-sample equal

in weight to one-tenth of the total material and to the original weight of a single zone (i.e. 0·09 gm.). This sub-sample was *cut transversely* into as fine a series of fragments as possible, subjecting the whole sub-sample to this treatment *throughout the length of the fibres* composing it. The fragments were poured off and the wool allowed to dry. The clump of wool fragments thus obtained was used to prepare a series of ten consecutive slides.

Before further treatments were commenced the ten zones into which the original material had been divided were allotted at random to two sections to facilitate the application of these methods of cutting, particularly Treatment D.

Treatment B: The two sections were placed adjacent to each other within the folds of an ordinary sheet of writing paper and cuttings made *transversely*, once in each of *three places* along its length, base, middle and tip. The fragments, which were cut as finely as possible (a little over 1 m.m. long) were mixed thoroughly in ether as before and then allowed to dry.

Treatment C.—In this treatment the two sections were *cut once transversely* about the middle of the staple and therefore adjacent to the central cutting of Treatment B. The fragments were mixed thoroughly and treated as before.

Treatment D.—In this treatment *a single oblique* cutting was made across the base half of one section and the tip half of the other and the fragments treated as above.

The clump of fragments finally obtained from each treatment was divided into ten approximately equal portions and from each portion a slide was prepared.* In the preparation of the slide each portion was divided into eight zones. From each zone a suitable quantity of fragments was drawn and carefully shaken out over the slide so that fragments from each zone were contributed to every part of the final preparation. The quantity drawn from each zone was completely used so that the question does not arise that the process of shaking the fragments over the slide tends to favour the extent to which either the coarser or finer fibres are contributed. With suitable care and experience slides can be prepared in this way in which no undue clumping of fragments is evident. Each slide was previously prepared by making a thin smear of the mountant, "Euparal", over the surface in the manner of a blood film. This was done so that the fragments falling on the slide would be *in situ* during preparation and as the cover slip was being pressed over the mountant. Such a precaution was taken because it had been noticed that when pressure was placed on the cover-slip as it was being set in place over the fluid mountant there was a tendency for fragments to be displaced towards the edges thereby disturbing their original even distribution over the slide. Cover slips measuring 2 in. by $\frac{7}{8}$ in. were used throughout and this constituted the area considered in the measurements. Slides prepared in this way may be retained as permanent preparations.

The readings from each slide were made in five longitudinal traverses, consisting of two series of twenty-five consecutive readings separated by a suitable interval. The readings were recorded individually in ten columns of twenty-five which could alternatively be considered as twenty-five rows of ten. Each slide was measured according to the same system by each observer but no attempt was made to make identical traverses. Certain eliminations were consistently made from the series to be measured. No obviously damaged or distorted fragments were considered nor were any tangled clumps, unless a very clear image presented itself. Crossed fibres were not measured if the point of intersection crossed the central section of the scale unless the image of the uppermost fibre could be very clearly distinguished. No fibre was measured whose image for one reason or another was not clearly defined.

In making a fibre measurement only those fibres, and that point of a fibre which passed across the central divisions of the ocular scale between the 20 and 30 unit lines, were taken for measurement. This procedure which constituted the ultimate sampling process, tended to eliminate personal selection and to bring the requirements nearer to the idea of random selection required by theoretical considerations.

Ordinary microscopes with the usual mechanical stage fittings were used by both observers throughout. The unit of measurement employed was $2{\cdot}5\mu$, at a magnification of $500\times$ and the setting of the microscope at this level was repeatedly checked against a Leitz stage micrometer.

The systems of recording the actual measurements used by the two observers were found to differ slightly. In both cases the division lines on the ocular were used to represent the means of the class intervals. But in the case of the observer P any observation clearly falling between the lines was classed as an intermediate measurement without any attempt at approximation to one division or the other. Such intermediate readings were allotted alternately to the higher and the lower class when the frequency distribution was subsequently drawn up. In the case of the observer Q, however, the only intermediate measurements recorded were those in which an approximation to one unit or the other could not be made. These intermediate readings were dealt with according to the common system which was employed in constructing the frequency distribution tables.

Methods of Statistical Analysis.

The statistical analysis of wool fibre diameter measurements is theoretically complicated by the fact that these measurements are by no means normally distributed. In the second study of this series (Malan, 1937) it was shown that the characteristic distribution of fibre diameter measurements is adequately represented by a $\log_e$ normal curve which is based on the assumption that the logarithms

of such measurements are normally distributed. On this basis the normal theory, strictly speaking, is not applicable to the actual measurements but to their logarithms.

It is not intended to discuss in detail the distribution of fibre diameter but only to illustrate the general form by two figures I and II. On these charts are presented the observed frequency histograms and best fitting logarithmic and normal curves. The histograms are those obtained from the second series of readings by the two observers P and Q respectively. Each histogram represents 2,500 measurements. The lack of normality is clearly shown on these charts by comparing the histogram with the normal curve indicated by the broken line. The improved fit of the $\log_e$-normal curve, shown by the continuous line, is equally clear on both charts. These charts represent very well the observed frequency distributions of fibre diameter measurements.

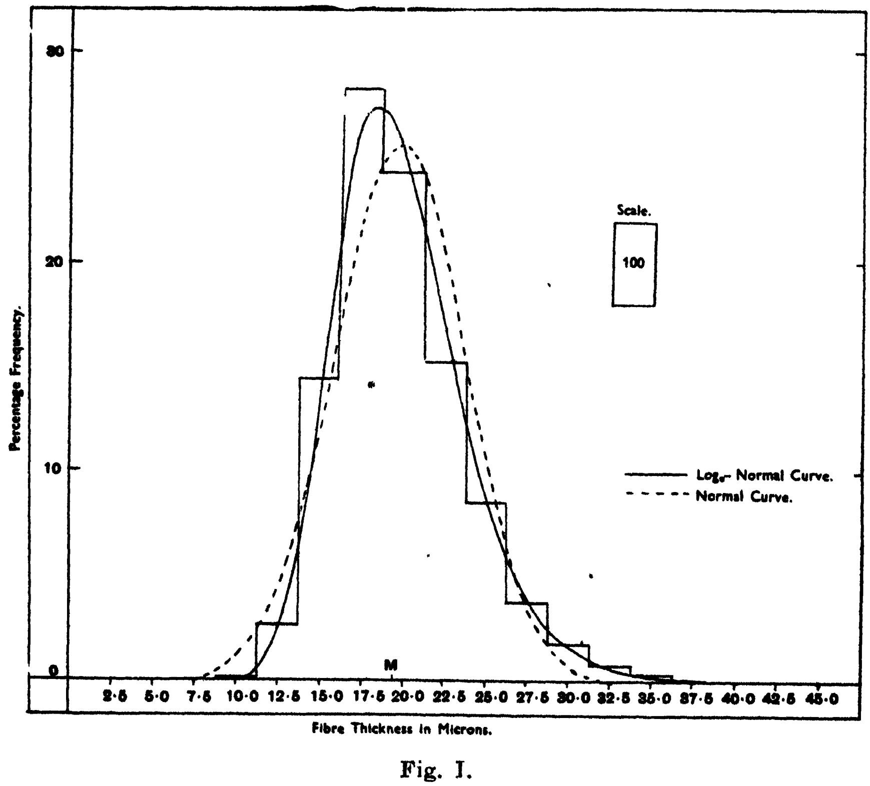

Fig. I.

The application of the normal theory to thickness measurements is more or less in general practice and it was thought advisable to include both the normal and logarithmic analyses of the data under discussion. This is done throughout the paper except for the analysis of variance within slides where the uniformity of distribution of the

fragments is considered. Here the group members are rather small and a transformation into logarithmic values by the method of moments becomes too inaccurate.

In the case of the logarithmic analyses the estimates of variance were calculated separately and not by subtracting sums of squares as is often done in the ordinary variance analyses. The reason for this is obvious since the variances (and means) are obtained by transformations of ordinary moments (Malan 1937) and any inaccuracy will seriously affect the difference sum of squares if one or more of the sums of squares is based on a small number of degrees of freedom. In such cases the logarithmic sums of squares were calculated from the logarithms of individual values.

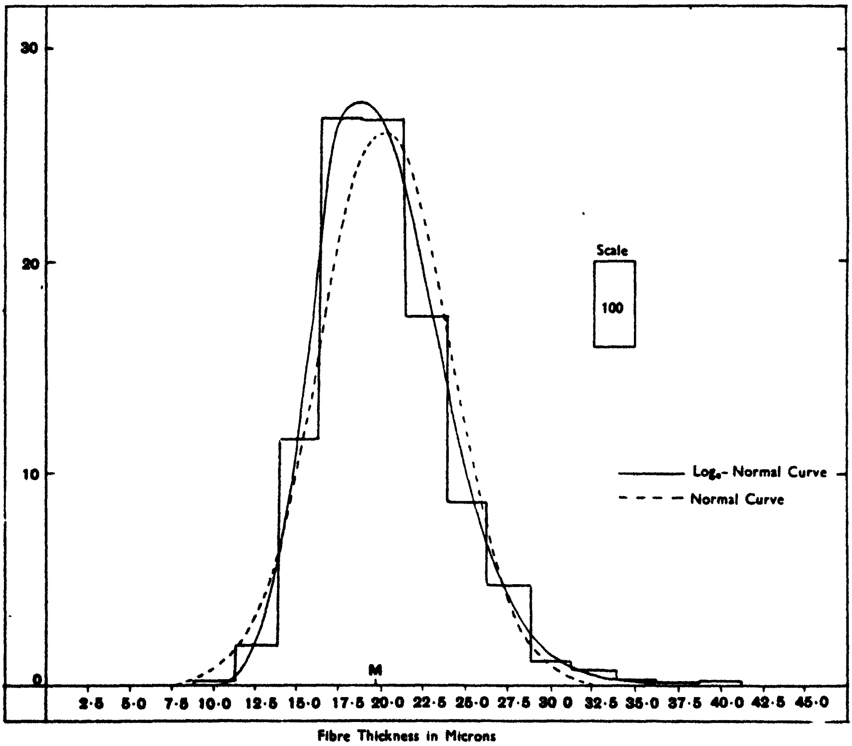

Fig. 11.

Presentation of the Data.

(a) *Variation within Slides.*

The observations from each slide were analysed as if they constituted a 10 by 25 Latin square. The variance between column means gives an estimate of uniformity in the sense that its significance would indicate a real difference between measurements from

different localities on the slide and hence a lack of uniformity in the distribution of fibre fragments. A full table of the analyses of variance is given in Table I. The variance for the two observers, P and Q, are given in adjacent columns for each slide separately. Table I (*a*) contains the results for the slides from Treatment A and similarly I (*b*), (*c*) and (*d*) present the results from each of the other treatments.

An examination of this table is sufficient to illustrate the satisfactory distribution of fibre fragments over the slides. When the row- and column-variances are compared with the corresponding error or remainder variance a significant value is indicated in italics for the 5 per cent. probability and in black type for the 1 per cent. or " highly significant " probability level.

The analysis of variance within each slide contains two independent comparisons of variance, viz. the variance between groups of 25 (columns) and that between groups of 10 (rows) with the remainder variance. Therefore, since there are four treatments with the ten slides each and two observers, the total number of comparisons is 160. As the same slides were measured by both observers there is reason to believe that their results will not be entirely independent. It should be remembered, however, that each slide contains many more fibre fragments than the 250 required for measurement and that no endeavour was made by the observers to measure the same localities on each slide. It is extremely unlikely therefore that the row and column variances will be highly correlated as between observers. Any agreement in this regard between observers should consequently be considered as more evidence that such a result reflects the position on the slide as a whole and not only as describing a particular set of observations from the slide.

On the basis of 160 different comparisons significance entirely due to random sampling should be shown by a number of comparisons not greatly different from 8. Of the significant values, about two should be highly significant on the above assumptions. The actual position revealed by Table I is 12 significant values of which 3 are highly significant. The increased number of significant values is hardly indicative of a serious degree of heterogeneity in the fibre distributions within slides. No undue increase in variation between either columns or rows are shown by 29 of the 40 slides. Of the remaining eleven where significance is shown, there is one case in which rows were effected and two others where the variance between columns was less than the " error " variance. This is evidently due to chance. In the case of the other eight slides the increased variation between columns was only shown by one or other of the two observers, except for one slide (No. 10) of Treatment D where the estimate of variance between columns is significantly greater than the " error " variance for both observers.

In Table I is also given a column which combines the degrees of freedom from all the slides for each treatment and observer separately. The respective degrees of freedom in this column are

in fact ten times the corresponding degrees of freedom for individual slides. These numbers are beyond the available tables and the estimates of variance are compared by calculating their standard errors and the standard errors of their differences. Only in the case of Treatment D is there, for both observers, a significantly increased estimate of variance between columns as compared with the error variance. These differences between the estimates of variance are for observer P, $6 \cdot 288 \pm 2 \cdot 2905$; i.e. approximately $2 \cdot 75$ times its standard error, and for observer Q, $5 \cdot 083 \pm 2 \cdot 407$, i.e. $2 \cdot 1$ times its standard error.

It may therefore be concluded that the preparation of some of the slides of Treatment D was less efficient than may be expected in the sense that the fragments were not uniformly distributed over the slide. For the other treatments the distribution of fragments was on the whole quite satisfactory. The lack of uniformity in the spread of fragments in Treatment D may indicate that the oblique method of cutting demands special care when slides are being prepared. It was in fact more difficult in this treatment to cut fragments as short as was possible with the other treatments and this in itself may explain the defect noticed.

(b) *Representativeness of Slides.*

The readings from the ten slides prepared from each of the four treatments were used to determine the variation between slides prepared consecutively from the same mixture of fragments. This variation indicates the uniformity of such a mixture. A separate analysis of variance between and within slides was made for each treatment and observer, the results being given in Table II (*a*) and (*b*) for the ordinary and logarithmic values respectively. The greatest difference in the variances between slides and the corresponding variances within slides is shown in the readings of observer Q for Treatment D where the estimates differ by a quantity about $1 \cdot 86$ times its standard error. This result which is the same for the ordinary and the logarithmic figures, is insignificant, and the results in general therefore illustrate the reasonable agreement between slides from the same treatment.

The arithmetical and geometrical mean diameters, as estimated from each slide, for observers separately, are presented in Table III (*a*) and (*b*) respectively. In treatment D, slide 4, the mean value obtained by the observer Q is rather lower than the others but this may be a chance effect since the variations between all slides is not significantly greater than the variation within slides. It should, however, be noted that the variance between slides is reduced by ignoring slide 4 to a value approximately equal to the estimated variance within slides. In any case the slide means for a particular treatment and observer are in satisfactory agreement amongst themselves.

TABLE II.

Analysis of Variance between and within Slides.

(a) Ordinary values.

Variance due to.	D.F.	A.		B.		C.		D.	
		P.	Q.	P.	Q.	P.	Q.	P.	Q.
Between slides	9	8·390	21·057	20·372	14·720	18·694	8·863	10·413	29·614
Within Slides	2,490	14·712	16·005	15·058	14·599	13·320	13·617	15·441	15·859
TOTAL	2,499	14·689	16 023	15·077	14·600	13·339	13·600	15·423	15·909

(b) Logarithmic values.

Variance.	D.F.	A.		B.		C.		D.	
		P.	Q.	P.	Q.	P.	Q.	P.	Q.
Between Slides	9	0·02065	0·04749	0·05391	0·03309	0·02709	0·02158	0·01932	0·06964
Within Slides	2,490	0·03699	0·03832	0·03794	0·03569	0·03042	0·02990	0·03724	0·03745
TOTAL	2.499	0 03709	0·03809	0·03809	0·03571	0·03053	0·02977	0·03813	0·03760

Table III.

Mean Values in μ for Treatments A, B, C and D and Observers P and Q.

(a) Arithmetical Means.

Slide.	A.		B.		C.		D.	
	P.	Q.	P.	Q.	P.	Q.	P.	Q.
1..........	19·79	20·39	20·12	20·37	20·81	21·39	19·85	20·61
2..........	19·73	20·29	19·44	20·11	21·08	21·10	19·74	20·28
3..........	19·49	20·10	20·00	19·82	20·30	21·16	20·27	20·12
4..........	19·89	20·41	19·46	19·64	20·87	21·19	19·82	19·51
5..........	19·72	19·64	20·04	20·16	20·72	21·10	19·82	20·66
6..........	19·81	19·91	19·49	20·38	20·80	21·06	20·12	20·68
7..........	19·35	20·00	19·36	19·83	20·66	21·49	19·73	20·40
8..........	19·60	20·53	19·49	19·87	20·81	21·37	19·88	20·25
9..........	19·93	20·06	19·68	20·09	20·55	20·93	20·27	20·31
10..........	19·83	20·51	19·85	20·09	20·60	20·95	19·96	20·49
Mean........	19·71	20·18	19·69	20·04	20·72	21·17	19·95	20·33

(b) Geometrical Means.

Slide.	A.		B.		C.		D.	
	P.	Q.	P.	Q.	P.	Q.	P.	Q.
1..........	19·44	20·03	19·76	20·05	20·53	21·12	19·50	20·28
2..........	19·35	19·98	19·10	19·75	20·79	20·79	19·47	19·95
3..........	19·15	19·71	19·68	19·49	20·02	20·90	19·87	19·77
4..........	19·53	20·01	19·11	19·35	20·60	20·83	19·43	19·16
5..........	19·37	19·35	19·63	19·75	20·34	20·82	19·48	20·27
6..........	19·44	19·55	19·08	20·04	20·46	20·78	19·73	20·22
7..........	19·04	19·59	19·08	19·54	20·31	21·18	19·40	20·04
8..........	19·24	20·15	19·09	19·55	20·55	21·06	19·54	19·93
9..........	19·58	19·68	19·28	19·69	20·26	20·67	19·84	19·90
10..........	19·50	20·08	19·53	19·74	20·33	20·58	19·57	20·13
Mean........	19·36	19·81	19·33	19·69	20·39	20·87	19·58	19·96

The analyses of the ordinary and logarithmic variances do not differ materially and, in fact agree very closely on the results of the significance tests. When the two types of variance are considered it should be realised that the ordinary standard deviation is measured in units of observation while the corresponding logarithmic coefficient is a measure of relative variability. When this coefficient is multiplied by two it has been termed the coefficient of relative variability and is, to some extent comparable with the ordinary coefficient of variability. These data are given in Table IV (*a*) and (*b*) respectively.

TABLE IV.

Coefficients of Variability.

(a) Ordinary Values.

Slide.	A.		B.		C.		D.	
	P.	Q.	P.	Q.	P.	Q.	P.	Q.
1..........	18·95	18·94	19·14	17·95	16·54	16·01	19·11	18·05
2..........	19·93	17·65	19·05	19·23	16·78	17·24	16·70	18·22
3..........	18·93	19·98	18·02	18·44	16·83	15·98	20·13	19·07
4..........	19 48	20·18	19·12	17·45	16·39	18·97	20 22	19·09
5..........	19·18	17·30	20·48	20·47	19·35	16·42	18·74	19·82
6..........	19·60	19·38	20·94	18·47	18·43	16·47	20·57	21·43
7..........	18·19	20·62	17·30	17 41	18·63	17·18	18·65	19·13
8..........	19·52	19·60	20 48	18·31	16·04	17·31	18·87	17·94
9..........	18·98	19·81	20·89	20·35	17·07	16·05	20·83	20·37
10..........	18·38	21 55	18 33	18 86	16 26	19 72	20·01	19·11
Mean........	19·10	19·61	19·37	18·73	17·28	17·08	19·36	19 30

(b) Logarithmic values.

Slide.	A.		B.		C.		D.	
	P.	Q.	P.	Q.	P.	Q.	P.	Q.
1..........	18·79	18·77	18·97	17·81	16·43	15·91	18·94	17·91
2..........	19·69	17·51	18·89	19·06	16·66	17·11	16·54	18·07
3..........	18·79	19·79	17·87	18·28	16·72	15·88	19·93	18·90
4..........	19·16	19·97	18·95	16·64	16·28	18·63	20·02	18·92
5..........	19·00	17·18	20·27	20 25	19·17	16·32	18·58	19·63
6..........	19·42	19·21	20·72	18·27	18·28	16·36	19·77	21·19
7..........	18·04	20·40	17·17	17·29	18 46	17·06	18 49	18 96
8..........	19·33	19·42	20 27	18·16	15·94	17·18	18·71	17·80
9..........	18·17	19·62	20·18	20·14	16·95	15·95	20·60	20·17
10..........	18·04	20·62	18·18	18·70	16·15	18·96	19·81	18·94
Mean........	18·84	19 31	19·18	18·49	17·14	17·26	18·91	19·08

(c) *Difference between Treatments.*

It was made clear in the description of the experimental layout that the difference in the treatments of the fibres before the actual preparation of the slides constituted a difference in the fibre populations sampled. The treatment means, although belonging to the same small staple do not, therefore represent identical fragment populations. This fact is further considered and illustrated by Table V which presents the analysis of variance between treatments, between slides, within treatments, and within slides for observers and methods of analysis separately.

TABLE V.

Analysis of Variance between Treatments.

(*a*) *Ordinary Values.*

Variance.	D.F.	Observers.	
		P.	Q.
Between Treatments	3	580·024	649·436
Within Treatments	36	14·468	18·564
Between Slides	39	—	—
Within Slides	9.960	14·633	15·020
TOTAL	9.999	—	—

(*b*) *Logarithmic Values.*

Variance.	D.F.	P.	Q.
Between Treatments	3	0·65213	0·70051
Within Treatments	36	0·03024	0·04295
Between Slides	39	—	—
Within Slides	9.960	0·03565	0·03534
TOTAL	9.999	—	—

The estimates of variance between treatment means is considerably higher than the other two estimates and there can be no doubt about the existence of real differences between them. These mean values are given at the bottom of the columns in Table IV (*a*) and (*b*) each being the result obtained from 2,500 observations.

The individual differences between the arithmetical and logarithmic values of the treatments means are further analysed in Table VI (*a*) and (*b*) respectively. Significant differences are printed in italics while black type denotes that the difference is highly significant. Thus it is seen that the means for Treatment C are highly significantly greater than those for the other treatments. The differences between the values for Treatments A and B are insignificant. The means of Treatment D occupy an intermediate position being less than C and greater than A and B. In the case of observer Q the difference between D and A is not quite significant.

The difference between the treatment means is adequately explained by variations in diameter along the length of the fibres composing the original staple. The relatively high value for Treatment C is probably due to the presence of a region of greater average

Table VI.

Differences between Treatment Means (in μ).

(a) Arithmetical Means.

		C.	D.	A.	B.
C.	P..................	—	**0·769**	**1·001**	**1·002**
	Q..................	—	**0·843**	**0·990**	**1·391**
D.	P..................	—	—	*0·232*	*0·253*
	Q..................	—	—	0·147	**0·295**
A.	P..................	—	—	—	0·021
	Q..................	—	—	—	0·148

(b) Geometrical Means.

		C.	D.	A.	B.
C.	P..................	—	**0·81**	**1·03**	**1·06**
	Q..................	—	**0·91**	**1·06**	**1·18**
D.	P..................	—	—	*0·22*	*0·25*
	Q..................	—	—	0·15	*0·27*
A.	P..................	—	—	—	0·03
	Q..................	—	—	—	0 12

thickness towards the middle of the staple, since this treatment represents a single transverse cut. The lack of a real difference between the values for A and B indicates that the changes in diameter along the length of the staple were proportionately represented by the three transverse cuts of Treatment B. Under normal conditions no real difference between A and D as regards mean fibre diameter was expected and the slightly higher mean for Treatment D is probably due to the removal of relatively narrow zones from the tip and base of the staple during the earlier treatments. These portions would ordinarily be included but in the present study this could not be done for treatment D owing to the exigencies of the special handling of the material during the process of preparation described earlier in this paper.

It is interesting to note from Table IV that the variance coefficients for Treatment C are less than the others. This corresponds to the fact that variations along the length of the staple were excluded by this treatment. Taking 17·2 per cent. and 19·0 per cent. as the coefficients of relative variability for Treatment C and the average for the other three respectively, it is found that the coefficient of relative variability *within* fibres is approximately 2·55 per cent. Within treatments the coefficients of variability estimated from different slides are in good agreement.

The Difference between Observers.

By considering the treatment means in Table III there is also, apart from the difference between treatments, an obvious difference between the corresponding means for the two observers. These differences are shown in Table VII (*a*) and (*b*). The treatment means for observer Q are consistently higher than those for P with an average difference between the arithmetical means of $0 \cdot 413 \pm 0 \cdot 0244\ \mu$ which is approximately 17 times its standard error. For the geometrical means the average difference is $0 \cdot 42\ \mu$, while the average difference between the natural logarithms of the geometrical means is $0 \cdot 0216 \pm 0 \cdot 002664$, i.e. about $8 \cdot 1$ times its standard error. (The standard errors are obtained from the variance within slides in Table V by the formula,

$$\text{S.E of difference} = \sqrt{\frac{S_P^2}{n_1} + \frac{S_Q^2}{n_2}}$$

where S_P^2 and S_Q^2 are the respective variances for the two observers P and Q and $n_1 = n_2 = 10{,}000$).

Since different microscopes were used by the two observers it was decided to include some further observations in which the personal and microscopic differences were separated, however unlikely a microscopic difference appeared. For this purpose the ten slides of Treatment A were chosen and divided into two random groups of five. One group was alotted to each microscope and all the slides were read by each observer on the respective instruments. The observed mean values, both arithmetical and geometrical are shown in Table VIII, (*a*) and (*b*) respectively, where the two microscopes are denoted by M_1 and M_2. An analysis of variance is given in Table IX (*a*) and (*b*) for the ordinary and logarithmic values respectively. There is obviously no indication of a difference between microscopes for either of the two observers. The difference between observers remained unaltered and in fact, was remarkably constant throughout all the observations.

TABLE VII.

Mean differences between Observers.

(*a*) *Arithmetical means.*

Observer.	Treatment.				Mean.
	A.	B.	C.	D.	
P..............	19·71	19·69	20·72	19·95	20·02
Q..............	20·18	20·04	21·17	20·33	20·43
Difference.........	0·47	0·35	0·45	0·38	0·41

(b) *Geometrical Means.*

Observer.	Treatment.				Mean.
	A.	B.	C.	D.	
P................	19·36	19·33	20·39	19·58	19·66
Q................	19·81	19·69	20·87	19·96	20·08
Difference.........	0·45	0·36	0·48	0·38	0·42
Ratio Q/P........	1·0232	1·0186	1·0235	1·0194	1·0219

TABLE VIII. (All values are in μ).

Slide means for the two microscopes and observers.

(Treatment A. New Series.)

(a) *Arithmetical means.*

Microscope.	Slide.	Observer.		
		P.	Q.	Q.–P.
M_1...........................	A_1.........	19·53	20·06	—
	A_3.........	19·45	19·60	—
	A_8.........	20·07	20·09	—
	A_9.........	19·88	19·76	—
	A_{10}.........	19·69	20·64	—
	Mean........	19·72	20·03	0·31
M_2...........................	A_2.........	19·70	20·16	—
	A_4.........	19·85	20·53	—
	A_5.........	19·86	20·13	—
	A_6.........	19·74	19·88	—
	A_7.........	20·29	20·12	—
	Mean........	19·89	20·16	0·27
	General Mean	19·81	20·10	0·29
M_1—M_2......................	—	−0·17	−0·13	—

(b) *Geometrical Means.*

Microscope.	Slide.	Observer.		
		P.	Q.	Q.–P.
M_1	A_1	19·17	19·75	—
	A_3	19·51	19·27	—
	A_8	19·68	19·78	—
	A_9	19·45	19·45	—
	A_{10}	19·35	20·27	—
	Mean	19·50	19·78	0·28
M_2	A_2	19·33	19·82	—
	A_4	19·43	20 19	—
	A_5	19·53	19·64	—
	A_6	19·34	19 52	—
	A_7	19 90	19·77	—
	Mean	19 41	19·75	0·34
	General Mean	19 44	19·76	0·32
M_1—M_2	—	0·07	0 05	—

TABLE IX. *Analysis of Variance.*
Comparison of Microscopes. (Treatment A. New Series.)
(a) *Ordinary Values.*

Variance.	D.F.	Mean Squares.	
		P.	Q.
Between Microscopes	1	16 800	11·900
Within Microscopes	8	14·970	26·568
Between Slides	9	15 173	24·938
Within Slides	2,490	15·417	14·566
TOTAL	2,499	15 471	14·601

(b) *Logarithmic Values.*

Variance.	D.F.	P.	Q.
Between Microscopes	1	0·00931	0·01095
Within Microscopes	8	0·02994	0·06683
Between Slides	9	0·02765	0·06062
Within Slides	2,490	0·03850	0·03399
TOTAL	2,499	0·03726	0·03439

Discussion.

In considering the methods of cutting employed in this investigation there is much to recommend Treatments C and D on statistical grounds. In these methods every fragment represents a different fibre whereas in Treatments A and B several fragments from the same fibre may be included in the same set of readings. This possibility is greater in A than in B since only three fragments per fibre could be included by the latter method, whereas in the former as many fragments as there were cuttings over the whole length of the fibres could be included. In view of the variations in thickness along the length of fibres it can hardly be determined how many cuttings per fibre and at which places would adequately represent the average fibre diameter of the sample. These objections are eliminated in Treatment D which contains fragments ranging over the whole length of the staple and at the same time only one fragment per fibre. Treatment D however requires great care in the spreading of the wool in an even layer thus to ensure that more or less the same number of fibres are cut at each point along the diagonal. By an uneven spread the number of fibres cut at different distances from the base will vary and the fragments will not properly represent the variations in thickness along the length of the fibre.

Treatment C presents the average diameter of the fibres at a particular stage of growth only and does not allow for variation in thickness along the length of the staple. This method will therefore give a smaller coefficient of variability, as the data shows, but the mean diameter will depend on the position of the transverse cut. For comparative purpose this method is the most useful provided there is no doubt about the position of the transverse line along which the sample is to be cut. Furthermore in view of the constant relation between the standard deviation and the mean fibre diameter for a particular sheep the genetical coefficient of variability is best obtained by Treatment C. • For the determination of this coefficient or variability the particular line of cutting is probably unimportant.

The data reveals a rather less satisfactory distribution of fragments in the case of slides from Treatment D which probably indicates that it is more difficult to obtain uniform mixtures when fragments are obtained by oblique cuttings. In this treatment it was certainly more difficult to secure cuttings as equal in length and as short as in the other treatments and good care should be taken in this respect. Length of fragment and evenness of length are probably the two most important factors in the preparation of a good mixture. The distribution of fragments over the slides of the other treatments was fairly satisfactory. Similarly the agreement between slides from the same mixture of fibre fragment was within the limits of random sampling in that the variation between such slides was in no case greater than the variation within slides.

The personal difference between observers was the only feature in the process of slide measurement which proved to be more serious than was originally anticipated.

The one observer obtained consistently lower mean values than the other. This feature is very disconcerting since it suggests that diameter measurements may only be regarded as strictly comparable when taken by the same observer. Even though the observed

difference was an extreme one in our experience, the possibility of its existence in other cases introduces an element of doubt in all comparisons where different observers are concerned. Observers from the same institute may be standardised but it is hardly possible to consider the standardisation of observers from different institutes and countries.

The methods of analysis based on the assumptions of normal and logarithmic distribution of fibre diameter measurements did not materially affect the results. The comparisons of variances seem to agree as regards significance when a probability level of 5 per cent. is taken. When the probability deviates considerably from the 5 per cent. level there appears to be a large difference between the two corresponding values of the respective analyses but this does not alter the conclusions since significance is judged by only considering the critical levels of 5 per cent. and 1 per cent. probability. It is suggested that the 5 per cent. probability level should be used for significance tests when the normal theory is applied. This level apparently agrees with the same level in the logarithmic analysis and there can be no doubt that the latter provides a more correct hypothetical distribution function for fibre diameter measurements.

It is to be noted that certain differences exist between the methods of slide preparation which we have adopted in this study and those advocated by Wildman and Daniels (1937). With us the fluid used for mixing was ether whereas Wildman uses cedar wood oil which is also his mountant. Ether may contain such impurities as water and alcohol which might affect the results by causing swelling of the fibres. While this point most certainly requires further investigation it does not affect the present study since the cuttings from the samples were mixed in the same sample of ether. Preliminary investigations with various samples of ether including some which were completely dry as well as others containing known volumes of water have not so far revealed any significant effect due to this factor. Another less important difference between Wildman's method and ours was in the final mountant used which in our case was "Euparal". The permanency of such a slide permits check measurements to be made when desired, since storage for long periods is possible.

This study has indicated that for samples of minimal size, (that is about 1 gram in weight) the measurement of a single well-prepared slide provides a satisfactory estimate of the mean fibre diameter. However, during the routine preparation of slides, cases inevitably occur where the sample it not adequately represented. In view of this we recommend the making of duplicate slides from each sample. Such slides will serve as a check on each other when required in doubtful cases.

As a check on the uniformity of dispersion of the fibre fragments on the slide we have found it useful to record the readings as successive groups of twenty-five, and to include the variance between such group means in the analysis of variance. Unsatisfactory slides may frequently be detected as a result of this procedure.

The present study is considered as a useful investigation preliminary to wider studies on the problems of sampling wool either in bulk or on the living animal.

Summary and Conclusions.

1. A series of fibre diameter determinations was made on a small staple of medium Merino wool, for the purpose of examining the representativeness of such measurements.

2. Four different methods of preparing the material were adopted and ten slides made from each.

3. Two observers measured 250 fibre fragments on each of the forty slides thus obtained.

4. The advantages of each method of treatment are separately discussed.

5. An unexplained but highly consistent difference in the measurements made by the two observers was noted and is regarded as requiring further examination.

6. For each observer separately the results as regards variation both between and within slides for each treatment showed the consistency required by statistical theory.

7. Statistical analysis of the results according to both the normal and logarithmic theories of distribution showed good agreement at the 1 per cent. and 5 per cent. levels of probability. Best agreement was demonstrated at the 5 per cent. level.

8. It was concluded that the measurement of a single well prepared slide will provide an adequate estimate of the mean fibre diameter of a wool sample of the size examined in this study. It is recommended however that in routine analyses permanent duplicate slides be prepared and that provision be made in recording the results for calculating the variance between successive groups of readings within the slides.

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Section IX.

Poultry Research.

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The Influence of the Protein Level of the Diet on the Growth, Egg Production, Egg-weight, and Mortality of Single Comb White Leghorn Pullets.

By J. J. BRONKHORST, Section of Poultry Research, Onderstepoort.

ALTHOUGH many papers have been published on this subject, it was felt that the question was important enough to warrant repetition using local foodstuffs. The rising mortality rate in poultry flocks all over the world is causing considerable anxiety, and very much is being written about the causes. In turn, almost every conceivable factor has been blamed for the present state of affairs. Dry mash feeding, forcing for high egg production, unnatural methods of management, the prevailing systems of breeding, and the hygiene of the farm have all had their share of criticism.

PLAN OF EXPERIMENT.

The nine weeks old Single Comb White Leghorn pullets were divided into three groups and an attempt was made to get them as equal as possible by dividing full sisters into the different groups. Where a particular hen did not have three daughters, half sisters were used to make the division as equal as possible. The pullets were not all hatched at the same time, but with the exception of the oldest lot of pullets, there was not much difference between them. As it happened, the first group had a few more of the oldest pullets, which accounted for the slightly higher production in February, and also the higher average egg weight.

The mash rations used are set forth in Table I.

TABLE I.

Mash Rations.

	Lot I.	Lot II.	Lot III.
Wheat Bran	20	20	20
Pollard	30	30	30
Yellow Maize Meal	40	35	30
Meat and Bone Meal	10	15	20

In addition to the above dry-mash ration, the birds had one feed of grain, viz. crushed yellow maize. The amount of crushed yellow maize was kept the same in all groups, the amount being that consumed by the birds which ate the least. The disadvantages of this method were realized but an attempt had to be made to keep the system of feeding as close as possible to the system in most common usage. Oyster shell and water were supplied *ad lib*, and the fowls had one feed of chopped green lucerne daily.

RESULTS.

The pullets were weighed at nine weeks and at fortnightly intervals thereafter. These weights are given in Table II, and represented graphically in Fig. 1.

TABLE II.

Bi-weekly Weights of Pullets in Pounds.

Age in Weeks.	Lot I.	Lot II.	Lot III
	lb.	lb.8	lb.
9	1·14	1·16	1·22
11	1·55	1·60	1 53
13	1·96	1·95	1·89
15	2 37	2·40	2·26
17	2·59	2·70	2·60
19	2·88	2·87	2 83
21	3·08	2·97	3 05
23	3·31	3·30	3·31

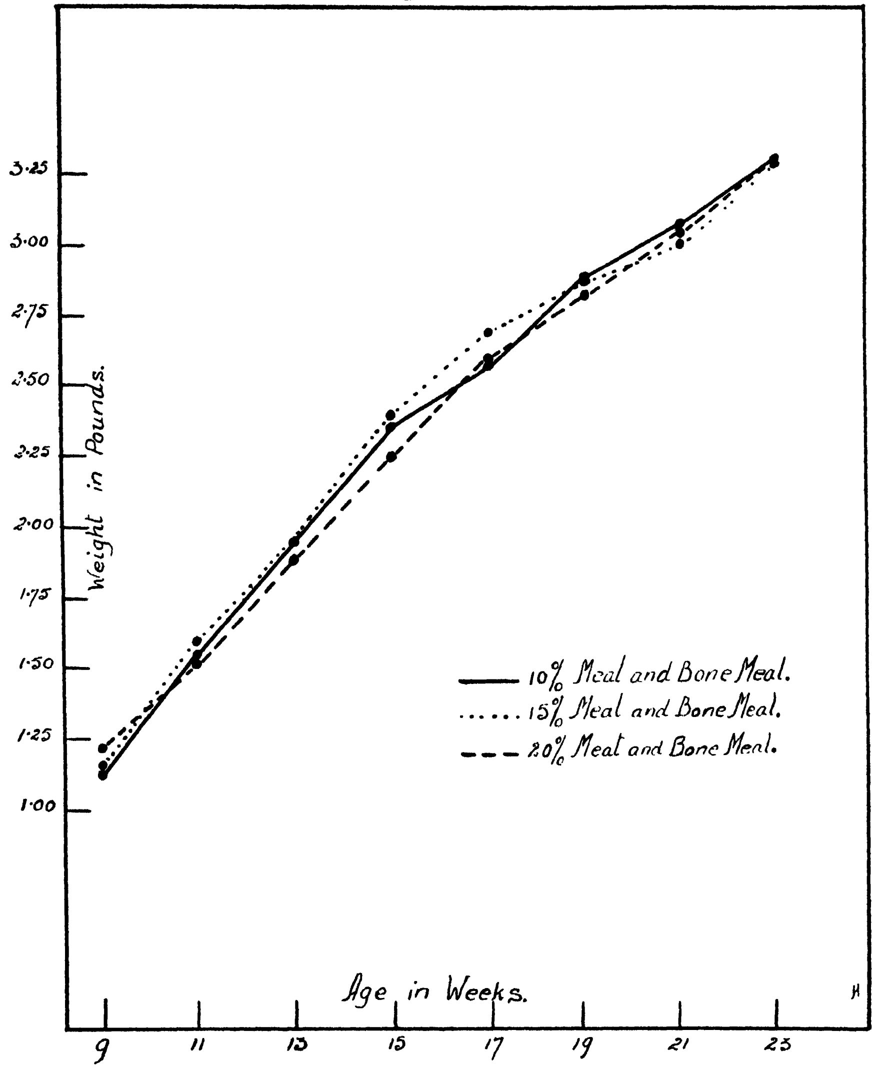
The only conclusion that can be drawn from Table II and Fig. 1 is that all groups grew at almost the same rate. This would indicate that the requirement for protein is lower after the age of eight weeks, and is in agreement with the work of Norris and Heuser (1930) St. John, Carver, Johnson and Brazie (1933), and McConachie Graham and Branion (1935).

SEXUAL MATURITY.

The value of the data on sexual maturity (date of first egg) was impaired by a rather severe outbreak of chicken pox. The onset of production was thus retarded in a number of individual cases. On a mash containing ten per cent. meat and bone meal, 63 pullets came into production at the average age of 192·7 days. On the mashes containing fifteen and twenty per cent. meat and bone meal, seventy pullets in each group matured at the average age of 201·6 and 201·5 days respectively.

The level of protein fed did not seem to influence the time of sexual maturity. Heuser and Norris (1933) also stated that the protein level influenced the time of maturity only to a small extent, except in cases where growth was definitely retarded by low protein levels. Winter, Dakin and Bayes (1932) state too that there was no correlation between the level of protein intake and the age at which the first egg was laid.

Fig. I.



EGG WEIGHT.

All eggs produced were weighed to the nearest eighth of an ounce. The object of weighing all eggs was to determine whether any differences existed which could be attributed to the rations, and also to find a more convenient and practical way of determining the annual average egg weights of pullets. Parkhurst (1933), in a rather extensive study of factors affecting egg weight, came to the conclusion that rations varying in protein content did not lead to the production of eggs that differed materially in weight. On the other hand, Heuser (1936) stated that a ration containing 14 per cent. protein was not conducive to the best egg size. Table III gives the monthly average egg weights, standard deviations and standard errors of the mean for the three groups.

TABLE III.

Monthly Average Egg Weights.

Month.	I.				II.				III.			
	No. of Eggs.	Mean.	S.D.	S. Em.	No. of Eggs.	Mean.	S.D.	S. Em.	No. of Eggs.	Mean.	S.D.	S. Em.
February	221	1·59	·136	·0091	217	1·57	·169	·0115	176	1·52	·120	·0090
March	294	1·77	·134	·0078	239	1·69	·164	·0106	466	1·65	·159	·0074
April	375	1·77	·161	·0083	415	1·83	·138	·0068	643	1·84	·128	·0050
May	880	1·89	·164	·0055	986	1·94	·156	·0050	1,020	1·94	·156	·0049
June	893	1·92	·151	·0051	1,006	1·96	·161	·0051	1,151	1·99	·156	·0046
July	975	2·00	·157	·0050	1,122	2·04	·155	·0046	1,306	2·07	·159	·0044
August	1,059	2·05	·142	·0044	1,348	2·07	·144	·0039	1,308	2·09	·150	·0041
September	987	2·06	·138	·0044	1,260	2·08	·152	·0043	1,235	2·10	·154	·0045
October	856	2·01	·143	·0049	1,235	2·08	·153	·0045	1,055	2·08	·168	·0052
November	743	2·03	·154	·0056	1,118	2·12	·147	0044	1,084	2·11	·176	·0053
December	661	2·05	·145	·0056	943	2·12	·143	·0047	954	2·14	·188	·0061
TOTAL	7,944	1·966	·187	·0021	9,889	2·023	·175	·0018	10,398	2·203	·205	·0020

From April to the end of the year, groups 2 and 3 produced heavier eggs than group 1. The difference between 1 and 2 is ·057 ± ·0029. The difference is 20 times as large as the Standard error of the difference, and there can thus be no doubt as to the significance of this difference. 15 and 20 per cent. meat meal in the ration produced eggs of the same size, but 10 per cent. caused a significant decrease in egg size.

Part-time Records of Egg Weight and Annual Average Egg Weight.

Several investigators have attempted to arrive at a suitable average annual egg weight by means of short time records.

Funk and Kempster (1934) found that the age and body weight at sexual maturity influenced the weight of the first ten eggs laid, but this was not closely correlated with the maximum and average egg weights.

In a study of Egg Laying Test records, Ginn (1932) found that a fairly reliable estimate could be obtained by weighing two eggs per month.

Godfrey (1933) stated that an approximation of the annual mean egg weight could be obtained from a knowledge of the average weight of the first ten eggs, the body weight when the first egg was laid, and the age at which laying commenced. A more reliable estimate was obtained by weighing all eggs laid the first four days of each month, and the best estimate was obtained by weighing the eggs on one specified day of the week throughout the first laying year.

Jull and Godfrey (1933) found that the minimum average of the first ten eggs, in order that birds should give standard eggs later on, varied with different flocks.

Wilson and Warren (1934) found that the month when pullets started to lay was important in estimating whether a bird would achieve the standard two ounce egg later. The later a bird starts to lay, the higher should be the average weight of the first ten eggs.

The average weight of the first ten eggs, and the estimate of egg size later, are important in cases where pullets are destined for egg laying tests. For ordinary breeding purposes, however, the disadvantage is that pullets do not start to lay at the same time, and egg weighing must thus be extended over three or four months. Furthermore, many pullets are inclined to lay a few eggs on the floor when they first start to lay. Weighing eggs for a few days a month or one day every week will also be rather a tedious procedure by the end of the year. To be of the greatest practical use, short period testing should be over as soon as possible and be done when most birds are in production.

In order to arrive at such a period the monthly average egg weight was correlated in South Africa with the annual average, and the regression formulae calculated. Table IV gives the coefficients of correlation, the number of individuals involved, and the regression formula in which X is the average egg weight for the year and Y is the average egg weight for the corresponding month.

TABLE IV.

Month.	Coefficient of Correlation.	n.	Regression formula.	$100r_2$.
May	·774 ± ·026	107	X = ·7356 Y + ·59	59·91
June	·778 ± ·023	123	X = ·8136 Y + ·42	60·53
July	·829 ± ·018	132	X = ·9436 Y + ·08	68·72
August	·901 ± ·011	135	X = ·928 Y + ·11	81·18
September	·877 ± ·013	134	X = ·8932 Y + ·17	76·91
October	·852 ± ·016	134	X = ·8458 Y + ·29	72·59
November	·892 ± ·012	134	X = ·892 Y + ·16	79·57
December	·858 ± ·016	128	X = ·782 Y + ·38	73·62

The monthly average weight is quite closely correlated with the annual average egg weight. The coefficient increases as the season advances and is at a maximum in August; from this time to the end of the year, there is an irregular decline.

The irregularity during early summer (November in South Africa) is probably due to climatic conditions.

The last column of Table IV gives the percentage variation in the total egg weight which can be established from average egg weights determined over periods of one month. Judging from this, one must conclude that either August or September or November in South Africa would be the best month to weigh eggs for computing annual average pullet egg weights.

EGG PRODUCTION.

The percentage production on a hen-day-basis from February to December is given in Table V and the same data are illustrated in Fig. 2.

TABLE V.

Month.	I.	II.	III.
February	15·1	15·3	12·6
March	20·6	15·9	32·4
April	17·3	17·6	27·3
May	42·0	40·4	43·8
June	45·7	42·9	52·8
July	50·5	47·4	57·8
August	59·5	59·6	60·2
September	63·5	62·3	62·1
October	60·7	61·5	55·3
November	53·4	60·3	60·3
December	58·2	56·0	56·8
TOTAL	43·6	44·5	48·5

Fig. II

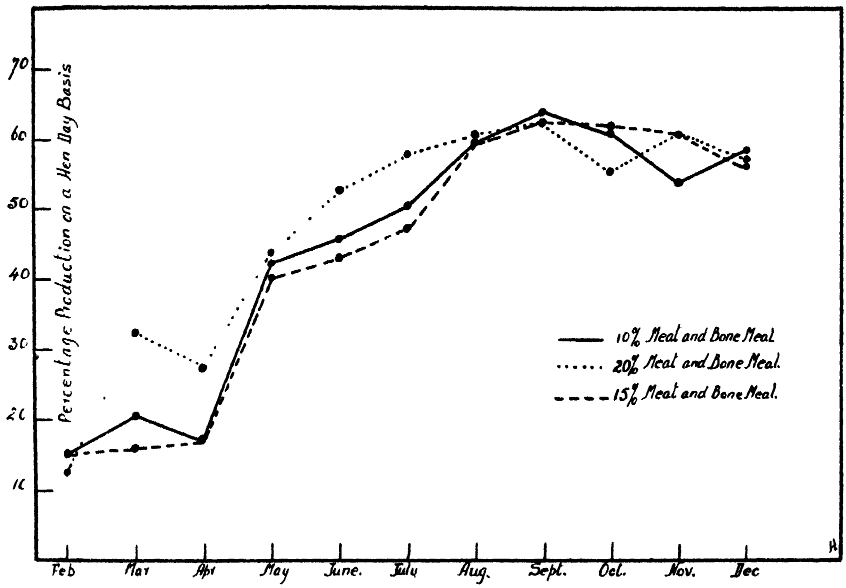


Table VI gives the average egg production, standard deviation, and standard error of the mean for all birds in each group which completed the experiment. Birds with records of less than 100 eggs were excluded due to the possibility of such birds suffering from some organic disease. Thus 5 birds in group 1, 10 birds in group 2, and 5 birds in group 3 were excluded. Among the birds excluded were a few that showed excessive broodiness, their low production being due partly at least, to a poor genetic make-up.

TABLE VI.

Group.	Mean.	Standard Deviation.	Standard Error of Mean.	No. of Individuals.
I..........................	159 5	22 8	4·03	32
II..........................	153 0	24 0	3 57	45
III..........................	163·0	33·6	4·66	52

An examination of Tables V and VI reveals no significant group difference in egg production. During March and April, there was a slight difference in favour of group 3, but the production of all groups was below normal then, due to an outbreak of chicken pox. It might have been that groups 1 and 2 were more susceptible, or that group 3 was more resistant to the virus. (At this stage it is of interest to consult the mortality records).

We conclude that under the conditions of this experiment, mash rations containing 10, 15 and 20 per cent. meat and bone meal, fed in conjunction with approximately equal quantities of crushed yellow maize, do not influence markedly the number of eggs produced by pullets during their first laying year.

Mortality.

Table VII gives an analysis of the mortality records. The most striking fact is the abnormally high death rate in each group.

Prolapse of the oviduct, with or without pickout, was the main cause of death. Many cases were found dead and eviscerated and it is presumed that cannibalism was incited by protrusion of the oviduct. In most cases, the birds were found before they were eviscerated, but these usually developed severe salpingitis and died or had to be killed, only a few recovering. Slightly more than 40 per cent. of the total mortality was due to this cause. Most interesting and important is a comparison of the prolapse-rates in the three groups. Very few cases occurred in the group receiving the highest percentage of meat and bone meal in the mash.

Table VIII suggests a seasonal incidence of prolapse, most cases occurring in spring. In group 1, two cases occurred immediately after the onset of laying. In group 2, one case occurred in April on the day the later hatched pullets were put in permanent quarters. Possibly this shifting exerted some influence. From July till the end of the year, this group had a regular mortality of two per month. In group 3, one early case occurred in May and the other two late in December.

Table VII.

Cause of Death.	10 Per cent. Meat and Bone Meal.		15 Per cent. Meat and Bone Meal.		20 Per cent. Meat and Bone Meal.	
	Number Died.	Percentage of total Mortality.	Number Died.	Percentage of total Mortality.	Number Died.	Percentage of total Mortality.
Prolapse of oviduct with or without pickout.....	22	56·4	13	48·1	3	12·5
Lymphoid Leucosis......	8	20·5	3	11·1	5	20·8
Erythroleucosis..........	—	—	—	—	1	4·2
Other tumours..........	2	5·1	2	7·4	1	4·2
Neurolymphomatosis.....	2	5·1	3	11·1	1	4·2
Yolk Peritonitis.........	1	2·6	1	3·7	1	4·2
Fatty degeneration of liver	—	—	1	3·7	2	8·3
Cysts of Mullerian Duct..	—	—	—	—	3	12·5
Accidental.............	—	—	—	—	2	8·3
All other causes..........	4	10·3	4	14·8	5	20·8
Total..........	39	100·0	27	99·9	24	100·0
Original No. of birds.....	78		82		78	
Percentage Mortality.....	50·0		32·9		30·8	

In these experiments the pullets were on their particular rations from the age of nine weeks. Were prolapse the result of a deficiency associated with the animal protein supplement, one would expect cases to occur first in the birds that were unable to build up reserves in their bodies before the onset of egg production.

TABLE VIII.

Month.	I.	II.	III.
February	2	—	—
March	—	—	—
April	1	1	—
May	1	—	1
June	—	—	—
July	3	2	—
August	4	2	—
September	6	2	—
October	3	2	—
November	2	2	—
December	—	2	2
TOTAL	22	13	3

Other factors which might exert some influence on the incidence of prolapse are: size of egg, rate of production, and age at first egg. The potential influence of these factors has been investigated as far as possible with the available data.

Table IX gives the average weight of the first ten eggs laid by all birds and those that suffered from prolapse. The birds in group 1 that suffered from prolapse laid slightly heavier eggs, but the difference is not significant. In group 2, the average weights were practically the same. In group 3, the three cases laid slightly smaller eggs, but the numbers are too small for comparison.

It would thus appear that egg size has no real influence on the incidence of oviducal prolapse. Only in one case, Hen A. 46, could prolapse be attributed to an abnormally large egg; protrusion accompanied the laying of the first egg which weighed 2·50 ounces. The average weight of the last egg laid by birds that died from prolapse was 2·01, 1·99 and 1·88 ounces respectively in the three groups. These weights do not differ materially from the annual averages given in Table III.

TABLE IX.

Prolapse and Egg Size.

Average of first 10 eggs.	I.	II.	III.
All birds	1·771	1·818	1·798
Prolapse birds	1·798	1·807	1·708
Difference	·027	·011	·090

The average age at sexual maturity, of birds with prolapse, was 190, 200 and 186 days for each group respectively. This does not seem materially different from the average ages at sexual maturity of all pullets, namely 192·7, 201·6 and 201·5 days for groups 1, 2 and 3 respectively.

The rates of production of birds suffering afterwards from prolapse was 59·3, 63·1 and 62·7 per cent for groups 1, 2 and 3 respectively. The rate was calculated from the time of the first egg to the time the prolapse occurred. Unfortunately these figures cannot be compared with any other general figure from the available data; however, they cannot be considered to be above the average, The idea of prolapse being due to exhaustion following heavy egg production would thus appear to be ill founded.

The populai literature always mentions cannibalism as being due to overcrowding; but in this case only 80 birds were placed in a house with 400 square feet of floor space (the regular allowance being 4 square feet per bird).

Other factors conceivably favouring the incidence of prolapse are tumours, weakness from any cause, spasms of smooth muscle, inflammation of the oviduct or cloaca, and an inherited predisposition.

When the affected bird is eviscerated by the fowls, the oviduct and intestines are lost and one cannot determine whether the bird was suffeiing from tumours or inflammation of the oviduct, etc.

Inherited factors were controlled as far as possible, each group having a full- or half sister. The hereditary make-up may play a rôle due to the interaction of genes and environment, but no confirmatory evidence of this was found.

Considering all the possibilities, the most plausible explanation of oviduct prolapse seems to be a nutritional deficiency. Perhaps an hereditary predisposition is also at work.

(Some families had more cases of prolapse than others but the figures were too small to permit definite conclusions.) The anti-prolapse factor seems to be associated with the protein supplement. Work to prove or disprove this is in progress.

Little need be said about the other cause of mortality, except that neoplasia were far too common. Infectious diseases and parasites were completely absent due to strict sanitation.

Conclusions.

1. The growth of pullets from nine weeks to maturity was the same on mash rations containing 10, 15 and 20 per cent. meat and bone meal, when fed in conjunction with crushed yellow maize as grain.

2. There was no significant difference in egg production which could be attributed to the different rations.

3. Birds receiving 15 and 20 per cent. meat and bone meal in the mash laid significantly larger eggs than birds receiving 10 per cent. meat and bone meal.

4. Prolapse of the oviduct occurred more frequently in the two lower protein supplement groups. The view is advanced that a nutritional deficiency, modified possibly by an hereditary predisposition, is responsible for the cases of prolapse.

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The Growth-Promoting Qualities of Various Protein Concentrates for Leghorn Chickens.

By J. J. BRONKHORST, Section of Poultry Research, Onderstepoort.

THE by-products of slaughter houses and of the fishing industry form very important ingredients in poultry rations. Not only is the protein supplement an expensive item, but it is extremely important in its influence on growth and reproduction, and may thus hold the balance between success and failure.

Most of the abattoirs in larger centres manufacture meat meal. In addition the fishing industry places various products on the market. Protein supplements from plant sources are not used as extensively for poultry as those from animals.

As yet nothing has been done to determine whether any marked differences exist between these South African products, which of them are most suitable, and if there is any way in which the quality of the products can be improved. One naturally expects that the materials used for protein supplements will differ widely, coming as they do from different sources, and the manufacturing processes being by no means uniform.

The literature on the biological value of proteins is enormous and it is not intended to give an extensive review, but rather to select a few articles with a direct bearing on the subject.

Hoagland and Snider (1926) reported poor growth on dried blood and serum protein, as compared with muscle protein. Ox palates, hog snouts, and pork cracklings also contained insufficient proteins for normal growth. In general, connective tissue seemed to be deficient in certain amino acids. Mitchell, Beadles and Kruger (1927) confirmed the statement that connective tissue seemed to be definitely inferior as far as the amino acid make-up was concerned.

Plimmer, Rosedal, Raymond and Lowndes (1934) reported on the relative values of various proteins, judged by the growth of chicks from 15-25 weeks of age.

THE GROWTH-PROMOTING QUALITIES OF PROTEIN CONCENTRATES FOR CHICKENS.

In a series of experiments, Prange, Hauge, and Carick (1927, 1928a, 1928b, 1928c) tested the efficacy of various protein supplements. One sample of meat meal was found to be definitely lacking in tryptophane, and commercial meat and bone scraps from different manufacturers did not give the same rates of growth when fed at the same protein level with mineral variations equalized. Tankage gave very poor growth with high mortality.

Titus, Byerly, Ellis and Nestler (1936) were of the opinion that the material used in the manufacture of good meat scraps and similar products was relatively more important than the temperature and the time of processing, as long as the temperature did not exceed 200 degrees F. or the time 8 hours. Higher temperatures were not studied.

Wilgus, Norris and Ringrose (1933) reported on the relative merits of various protein supplements, and placed them in the following descending order:—vacuum- and drum-dried fish meals, dried skim milk, domestic sardine meal, flame dried white fish meal, expeller process soybean oil meal, Asiatic sardine meal, steam dried menhaden meal, meat scrap, flame dried menhaden meal, hydraulic process soybean oil meal, whale meat, corn gluten, and blood. The vitamin G complex in these studies was of no account as the rations were all supplemented.

Daniel and McCullum (1931) emphasized the fact that general statements could not be made regarding the values of white and menhaden fish meals, since the values depended both on the raw materials and the methods of processing.

Maynard, Bender and McCay (1932), in balance experiments with rats found vacuum dried white meal to be superior to steam dried menhaden meal, and the latter superior to flame dried menhaden meal as regards growth promotion. The results suggested that differences in heat treatment were at least partially responsible for the nutritive differences found.

Almquist, Stokstad and Halbrook (1935) obtained their best results with vacuum dried beef and whale meat meal; meat scraps and cracklings were poorer and the tankages decidedly inferior. The authors suggested a formula, based on the analyses of intact protein, protein decomposition products, indigestible protein and hot water soluble protein, for the rapid laboratory determination of the quality of protein in a commercial concentrate.

Boas-Fixsen and Jackson (1932) mentioned the fact that caseinogen suffers a marked decline in biological value as the result of prolonged heating at 112° C.

Chick, Boas-Fixsen and Hutchinson (1935) gave further evidence regarding the heat injury of proteins. Heating caseinogen at 150° C. for 66 hours decreased the biological value from 66 to 44 per cent. and also decreased the digestibility from 93 to 73 per cent. The biological value of lactalbumen was only slightly reduced by heating at 120° C. for 72 hours, but the digestibility was lowered from 95 to 69 per cent.

Fairbanks and Mitchell (1935) also found a decrease in biological value of the best roller process milk powder due to preheating. This decrease is due to a partial destruction of cystine. If the temperature is increased further until scorching results, a further decrease takes place. The scorched products are improved by the addition of lysine. Digestibility is also impaired by scorching.

Further evidence concerning the effect of heat on proteins was given by Greaves and Morgan (1934). Heating casein for 30 minutes at 140° C. produced a definite change in the lysine and hystidine fraction of the protein, which resulted in a measurable lowering of its nutritive value for rats.

Morgan (1931) submitted cereal and casein proteins to toasting at approximately 150° C. for thirty minutes. In all cases a significant decrease in biological value was found. The digestibility of the toasted proteins was but little different from that of the raw, particularly when fed to older animals, and the inexplicable loss of N occurred chiefly in the urine, indicating that the change produced by the heat treatment lies probably in the assortment or availability of the amino acids absorbed.

In a later article, Morgan and Kern (1934) concluded that there appeared to be a heat injury to beef muscle protein, increasing in severity with the length of exposure and the height of the temperature reached.

Allerdyce, Henderson and Asmundson (1933) found that the results on four samples of Pilchard meals varied widely. The weight of the chicks at two weeks varied inversely with the fat content, but showed no relation to the protein and ash content. Putrefaction and high temperatures have a detrimental effect on the feeding values of fish meals, and this probably accounts for the differences which were found.

From an analysis of fish meals, Ingvaldson (1929) concluded that high temperatures (195° C.) caused an increase in humin and volatile basic N and a diminution in arginine N and cystine. The other constituents in the hydrolysates were unaffected. Since arginine and cystine are essential amino acids, and the amounts of these therefore help to determine the biological values of fish meals, heating to such high temperatures as 195° C. should be avoided in the preparation of these meals.

Maynard and Tunison (1932) prepared haddock waste and menhaden fish by both flame drying and vacuum drying, and the four resulting products were studied on rats by the N balance method. The protein of the vacuum-dried haddock proved superior to that of the flame dried product, in both digestibility and biological value. In the case of the menhaden products, a marked superiority in digestibility was shown for vacuum-drying, but the difference in biological value in favour of vacuum-drying was too small to be statistically significant. The big decrease in digestibility of menhaden products was explained by their high oil content, and the effect of the oil on the proteins present. The authors concluded that both heat and the source of the material influence the nutritive values of protein supplements.

Record and Bethke (1933) concluded that the fish meals commonly available on the market varied greatly in their nutritive values. These differences in nutritive value are probably due to variations in the biological values of the proteins, and in the vitamin G content of the meals as affected by the different methods of manufacture.

In a later paper, Record, Bethke and Wilder (1934) tested nine different samples of haddock and a sample of cod meat against meat scraps. Some samples were deficient in certain vitamins, which affected growth and gave rise to leg paralysis. When the samples were supplemented with liver extract or dried whey to supply the vitamin G complex, the differences disappeared. The protein values of the different fish meal samples were similar but both haddock and cod meal were superior to meat scraps.

In balance experiments with pigs, Schneider (1932) ranked fish meals in the following order, as regards the digestibility of their proteins: vacuum-dried white fish meal, steam-dried menhaden meal, flame-dried menhaden meal. In both experiments, the flame-dried meal was significantly inferior to the vacuum-dried meal.

Experimental.

Experiment I.

Single Comb White Leghorn chicks were used as experimental animals. In Experiments I and II, the chicks were taken from the pedigree baskets and wingbanded, and the chicks from each female were then distributed to successive lots, so that no full brothers or sisters were in the same lot, except in cases where a hen gave more than five chicks. The chicks for Experiment III were not pedigreed.

After banding, all chicks were placed in an electrically heated battery brooder, where they stayed for three weeks. They were then removed to another battery without artificial heat. Weekly records of food consumption and individual chick weights were kept. The first experiment comprised five lots, each lot getting their meat meal protein supplement from a different source. The analysis of the meat meals are given in Table 1.

TABLE I.

Supplement.	Moisture.	Ash.	Ether Extract.	Protein.
I	6·9	30·8	10·4	46·9
II	4·1	20·6	20·8	45·4
III	3·9	19·9	23·1	47·4
IV	6·9	20·8	11·2	62·1
V	4·7	27·5	15·3	45·1

The basal ration comprised yellow mealie meal, wheat bran, pollard and lucerne meal. The maize meal content was altered to make up the required amount after the addition of the protein supplement. Table II gives the actual rations fed, supplement I being given to Lot I and so on. The analyses of the rations are presented in Table III.

TABLE II.

Food.	Lot I.	Lot II.	Lot III.	Lot IV.	Lot V.
Yellow maize	49·0	48·0	49·5	55·5	48·0
Wheat bran	15·0	15·0	15·0	15·0	15·0
Pollard	10·0	10·0	10·0	10·0	10·0
Lucerne meal	5·0	5·0	5·0	5·0	5·0
Meat meal	20·0	21·0	19·5	13·5	21·0
Oyster shell	1·0	1·0	1·0	1·0	1·0
Salt	0·5	0·5	0·5	0·5	0·5
	100·5	100·5	100·5	100·5	100·5

TABLE III.

	Moisture.	Ash.	Ether Extract.	Protein.
Lot I	9·2	9·6	4·9	19·1
Lot II	8·5	7·3	7·9	19·0
Lot III	8·7	6·6	8·7	18·9
Lot IV	9·6	5·8	3·9	19·1
Lot V	8·7	9·0	6·6	19·5

TABLE IV.

Average Weekly Weights (in grams).

	Lot I.	Lot II.	Lot III.	Lot IV.	Lot V.
Day old	41·5	42·0	41·2	41·4	41·2
1 week	55·2	56·6	57·6	56·0	57·6
2 weeks	83·0	97·0	96·8	89·0	96·7
3 weeks	124·5	150·3	150·1	134·0	149·4
4 weeks	182·3	217·0	215·0	186·9	217·2
5 weeks	246·4	291·6	284·7	250·9	290·3
6 weeks	323·0	364·1	351·6	331·8	376·7
7 weeks	388·8	428·4	409·7	391·4	453·9
8 weeks Males	509·5	569·9	524·8	531·2	577·9
Females	441·1	454·5	443·1	431·2	486·3

Table V.

Food Consumption and Growth.

Week.	Lot I.			Lot II.			Lot III.			Lot IV.			Lot V.		
	Total food consumed. Grams.	Grams food per gr. gain.	Grams gain per gr. protein.	Total food consumed. Grams.	Grams food per gr. gain.	Grams gain per gr. protein.	Total food consumed. Grams.	Grams food per gr. gain.	Grams gain per gr. protein.	Total food consumed. Grams.	Grams food per gr. gain.	Grams gain per gr. protein.	Total food consumed. Grams.	Grams food per gr. gain.	Grams gain per gr. protein.
1st.....	2,125	2·36	2·22	2,080	2·36	2·23	1,725	2·35	2·25	2,035	2·23	2·35	2,115	2·09	2·45
2nd.....	4,793	2·50	2·11	5,504	2·27	2·32	4,110	2·33	2·27	4,798	2·35	2·23	5,345	2·24	2·29
3rd.....	7,398	2·65	1·98	5,985	2·47	2·13	6,145	2·56	2·07	5,315	2·41	2·17	8,386	2·61	1·97
4th.....	11,227	2·94	1·78	11,381	2·93	1·79	8,398	2·88	1·84	9,574	2·97	1·76	11,597	2·80	1.83
5th.....	14,418	3·40	1·54	13,437	3·31	1·59	10,055	3·21	1·65	11,933	3·05	1·71	14,383	3·16	1·62
6th.....	18,267	3·62	1·45	15,752	3·54	1·49	11,068	3·68	1·44	14,919	3·14	1·67	17,152	3·32	1·55
7th.....	19,265	4·51	1·16	14,935	4·10	1·28	12,075	4·61	1·15	16,090	4·50	1·16	18,240	4·06	1·26
8th.....	20,425	3·82	1·37	17,300	4·11	1·28	13,035	4·18	1·26	18,540	3·44	1·52	19,390	4·24	1·21
Total..	97,818	3·46	1·515	86,374	3·33	1·583	66,611	3·38	1·565	83,204	3·20	1·636	96,608	3·27	1·584

TABLE VI.

Health and Mortality.

Lot.	No. of chicks started.*	Paralysis.†	Mortality.	Percentage Mortality.	Percentage Paralysis.
I......................	67	1	3	4·5	1·5
II......................	62	0	7	11·3	—
III......................	45	1	1	2·2	2·2
IV......................	64	4	4	6·3	6·3
V......................	64	2	3	4·7	3·1

* The first night the chicks were in the electrically heated battery, a fuse blew, resulting in heavy mortality. The chicks that died due to chilling are not included in this column, and are treated as if they never started the experiment. This mortality accounts for the unequal numbers in the various groups.

† Paralysis means the paralysis of Norris (toes curled up and invariably turned inwards).

TABLE VII.

Differences in Mean Weight at Eight Weeks.

Lots.	Males.			Females.		
	Diff. grms.	T.	P.	Diff. grms.	T.	P.
1–2..............	60·4	3·50	< ·01	13·4	·79	> ·4
1–3..............	15·3	·81	> ·4	2·0	—	—
1–4..............	21·7	1·29	> ·2	9·9	·60	—
1–5..............	60·4	3·92	< ·01	45·2	2·84	< ·01
2–3..............	45·1	2·33	< ·05	11·4	·62	> ·6
2–4..............	38·7	22·2	< ·05	23·3	1·35	> ·1
2–5..............	8·0	·44	> ·6	31·8	2·06	< ·05
3-4..............	6·4	·34	> ·7	11·9	·66	·5
3–5..............	53·1	2·27	< ·05	43·2	2·48	< ·02
4–5..............	46·7	2·65	< ·01	55·1	3·37	< ·01

Table IV gives the average weekly weights of all chicks, and the average male and female weights at eight weeks of age. These data are presented graphically in Fig. 1.

Table V gives food consumption and growth data for the five lots, during the entire experimental period. The first column is the actual amount of food consumed, the second column, the amount of food required in grams to produce one gram of gain in live weight, and the third column, the gain in weight resulting from the ingestion of one gram of protein.

Tables VI and VII give the health and mortality records, and the differences between corresponding sexes, T values and probabilities, respectively.

Table VIII gives the average weights of chicks and the standard errors of chicks at eight weeks of age.

Fig. I.
Experiment I Growth Curves.

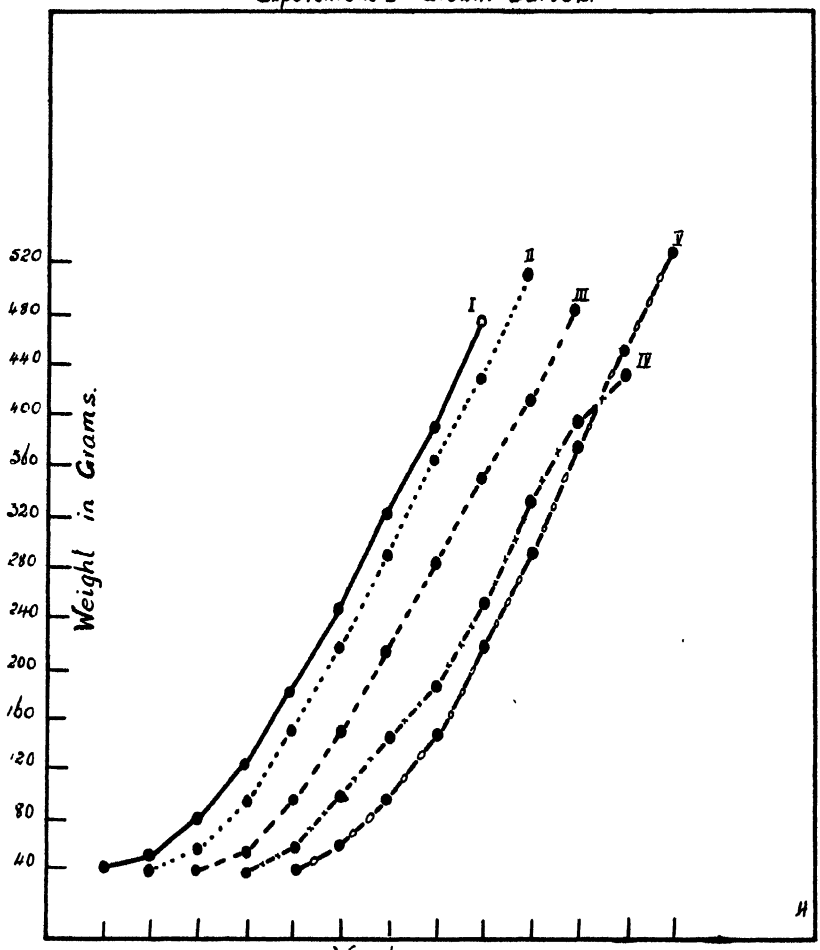


TABLE VIII.

Mean Weights and Standard Errors of Chicks at Eight Weeks of Age.

Lot.	Males.	Females.	Males and Females.*	No. of males.	No. of females.	Totals.
I....	509·5 ± 11·78	441·1 ± 11·42	474·2 ± 8·21	31	33	64
II....	569·9 ± 12·63	454·5 ± 12·40	511·1 ± 8·85	27	28	55
III....	524·8 ± 14·67	443·1 ± 13·39	480·2 ± 9·93	20	24	44
IV....	531·2 ± 11·98	431·2 ± 11·98	481·1 ± 8·47	30	30	60
V....	577·9 ± 12·87	486·3 ± 11·09	525·3 ± 8·49	26	35	61

* S.E. of unweighted mean live weight of males and females.

Discussion of Results.—This first experiment comprised four samples of meat and bone meal, of which the analyses were very similar as regards protein; the other sample, Lot IV, was considerably higher.

Samples II and III were from the same source as reflected by the similar ash and the very high ether-extract contents. Sample I was rather high in its ash content, and this was reflected in the composition of the ration; sample V was very similar in this respect.

The growth curves in Fig. 1 indicate the superior growth of lot V, lots I and IV revealing much slower growth, and lots II and III more moderate growth. From an inspection of differences and probabilities in Table VII, it can be seen that for the males, significant differences exist between lot V and all other lots except II, this lot in its turn being significantly superior to all others except V. The better growth of lot V is also reflected in the females, there being significant differences between the females in lot V and all others including lot II. The superiority of the males of lot II is not duplicated by the females in this group. It would thus appear that lot V was outstanding as far as growth was concerned, with lots, II, III, I and IV following in order.

In interpreting the results on the basis of food consumption per gram gain, and gain per gram protein ingested (Table V), a somewhat different picture is obtained. On this basis, lot IV appears to be best and lot I worst, while the rest are more or less similar.

The slight depression in the biological value of group V, which showed the best growth, when compared with group IV may be the result of the lower utilization due to the high food, and consequent protein, intake.

This method of estimating the biological value is used by Osborne, Mendel and Ferry (1919). The explanation of the slow growth of lot IV is probably to be found in the absence in the protein supplement of some growth promoting factor, not protein.

Table VI gives the mortality figures and incidence of leg paralysis of nutritional origin. It will be noticed that the incidence of paralysis was highest in lot IV; a sub-clinical manifestation of this deficiency might thus have been the cause of the slower growth, notwithstanding the higher biological value exhibited by this group as judged by the method of Osborne, Mendel and Ferry.

On the basis of the evidence presented, it is concluded that significant differences exist among the protein supplements tested. These differences may be due to the materials which are used in the manufacture of these products, or they may be due to differences in the manufacturing processes. The similarity in biological value for growth as measured by the method of Osborne, Mendel and Ferry, and marked differences in total growth would suggest that the manufacturing processes involving time and temperature of cooking, and temperature and duration of the drying process, play a very important part in the ultimate values of the finished products.

Experiment II.

The second experiment comprised one sample each of fish meal, crayfish meal, whale meal, pure meat meal, and meat and bone meal.

The analyses of these feeds are given in Table IX and the whole rations in Table X. The basal ration was the same as in Experiment I.

TABLE IX.

Analysis of Supplements.

Supplement.	Moisture.	Ash.	Ether Extract.	Protein.
I Fish meal	12·3	16·9	6·6	69·7
II Crayfish meal	6·9	30·3	4·8	40·5
III Whale meal	10·8	11·8	12·4	72·1
IV Pure meat meal	7·2	16·8	5·8	78·9
V Meat and bone meal	7·6	35·5	4·1	53·3

TABLE X.

Feed.	Lot I. Fish meal.	Lot II. Crayfish meal.	Lot III. Whale meal.	Lot IV. Meat meal.	Lot V. Meat and bone meal.
Protein supplement	11·5	23·0	11·0	10·0	16·0
Yellow maize meal	56·0	45·5	55·5	57·5	52·5
Wheat bran	15·0	15·0	15·0	15·0	15·0
Wheat pollard	10·0	10·0	10·0	10·0	10·0
Lucerne meal	5·0	5·0	5·0	5·0	5·0
Oyster shell	1·0	1·0	1·0	1·0	1·0
Salt	·5	·5	·5	·5	·5
Bonemeal	1·0	—	2·0	1·0	—
	100·0	100·0	100·0	100·0	100·0
Actual percentage protein	19·4	18·8	18·4	18·3	18·5

TABLE XI.

Average Weekly Weights.

	Lot I.	Lot II.	Lot III.	Lot IV.	Lot V.
Day old	40·4	40·8	39·9	39·9	40·3
1 week	51·3	53·2	54·4	55·0	52·5
2 weeks	63·8	71·2	85·0	80·0	74·9
3 weeks	80·5	97·4	126·9	114·1	100·7
4 weeks	101·3	132·6	173·9	150·0	137·2
5 weeks	128·5	180·7	228·5	194·0	184·0
6 weeks	155·3	231·7	280·6	237·0	232·0
7 weeks	191·6	293·1	345·0	294·6	296·7
8 weeks	242·4	378·2	420·8	365·7	376·9
Males	244·1	382·1	453·3	385·6	404·2
Females	240·2	372·2	392·5	355·0	356·4

TABLE XII.

Food Consumption and Growth

Week.	Lot I.			Lot II.			Lot III.			Lot IV.			Lot V.		
	Total food consumed. Grams.	Grams food per gr. gain.	Grams gain per gr. protein.	Total food consumed. Grams.	Grams food per gr. gain.	Grams gain per gr. protein.	Total food consumed. Grams.	Grams food per gr. gain.	Grams gain per gr. protein.	Total food consumed. Grams.	Grams food per gr. gain.	Grams gain per gr. protein.	Total food consumed. Grams.	Grams food per gr. gain.	Grams gain per gr. protein.
1 week.	1,465	2·97	1·74	1,470	2·70	1·97	1,475	2·25	2·42	1,490	2·25	2·42	1,640	2·92	1·85
2 weeks	2,475	4·42	1·17	2,670	3·46	1·54	3,040	2·30	2·37	3.090	2·80	1·95	3,060	2·97	1·82
3 weeks	2,975	3·96	1·30	3.665	3·26	1·63	4,510	2 44	2 23	4.210	2·81	1·95	4,175	3·51	1·54
4 weeks	3,795	4 20	1·23	5,080	3·35	1·59	6,055	2 93	1·86	5.420	3·43	1·59	5.565	3·31	1·63
5 weeks	4,300	3·72	1·39	6,600	3 19	1·67	7.135	2·97	1 83	6,785	3·48	1·57	7.065	3·45	1·57
6 weeks	5,185	4·49	1·15	8,005	3·65	1·46	8,245	3·84	1·42	7,625	4·06	1·34	8.860	4·11	1·32
7 weeks	4,990	4·42	1·17	9,913	3·95	1·34	9,525	3·44	1·57	9.000	3·80	1·44	10.310	3·67	1·47
8 weeks	6,380	4·19	1·23	12,060	3·37	1·58	10,825	3·32	1·64	10,875	3·59	1 54	12.165	3·50	1·53
TOTAL..	31,565	4·116	1·252	49,463	3·458	1·538	50,810	3·084	1·762	48.495	3·440	1·599	52.840	3·542	1·526

TABLE XIII.

Health and Mortality.

Lot.	No. of Chicks.	Paralysis.	Mortality.	Percentage mortality.	Percentage paralysis.
I	47	1	4	8·5	2·1
II	48	2	5	10·4	4·2
III	45	5	2	4·4	11·1
IV	45	8	2	4·4	17·8
V	45	4	1	2·2	8·9

TABLE XIV.

Differences in Mean Weight at Eight Weeks of Age.

Lots.	Males.			Females.		
	Difference in grams.	T.	P.	Difference in grams.	T.	P.
1–2	138·0	7·17	< ·01	132·1	6·55	< ·01
1–3	209·2	10·73	< ·01	152·3	7·63	< ·01
1–4	141·6	6·68	< ·01	114·8	6·00	< ·01
1–5	160·1	8·10	< ·01	116·3	5·85	< ·01
2–3	71·2	3·54	< ·01	20·2	1·05	·3
2–4	3·6	0·16	> ·8	17·3	0·94	·4
2–5	22·1	1·10	·3	15·8	0·85	·4
3–4	67·6	3·08	< ·01	37·5	2·07	·05
3–5	49·1	2·38	·05	36·0	1·96	slightly > ·05
4–5	18·5	0·83	·4	1·5	0·08	> ·9

TABLE XV.

Lot.	Males.	Females.	Males and Females.	No. of males.	No. of females.	Totals.
I	244·1 ± 13·142	240·2 ± 14·770	242·4 ± 9·885	24	19	43
II	382·1 ± 14·050	372·3 ± 13·726	377·1 ± 9·821	21	22	43
III	353·3 ± 14·396	392·5 ± 13·424	420·8 ± 9·842	20	23	43
IV	385·7 ± 16·623	355·0 ± 12·167	365·7 ± 10·300	15	28	43
V	404·2 ± 14·770	356·5 ± 12·626	376·6 ± 9·716	19	26	45

The average weekly weights and the mean weights of males and females are given in Table XI. A graphic representation of the growth of different groups is given in Fig. 2.

Table XII gives the weekly food consumption, efficacy of food utilization, and the gains per unit of protein ingested.

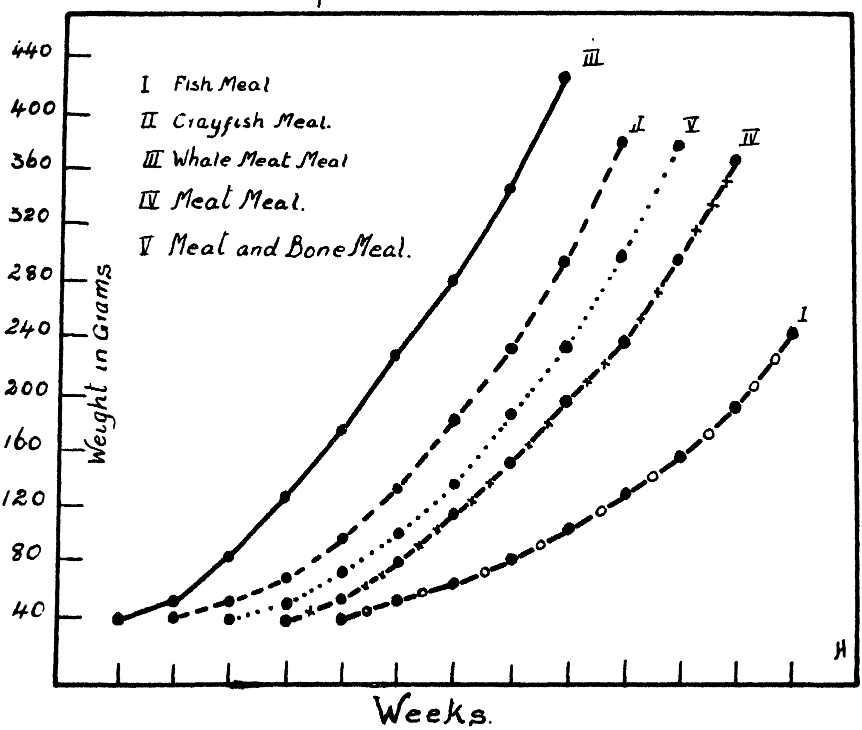
The health and mortality records are given in Table XIII, and the differences in mean weight at eight weeks of age, together with T values and probabilities, are given in Table XIV.

The mean weights and standard errors of males and females are given in Table XV.

Discussion of Results.—An attempt was made to stabilize the percentage protein at 19·0. Due to variations in the composition of the ingredients in the basal ration, lots III, IV, and V fell rather below this figure, notwithstanding the fact that every precaution was taken in sampling.

The most striking fact emerging from the average weekly weights (Table XI) and the growth curves (Fig. 2) is the extremely poor growth of chicks on fish meal.

Fig II.
Experiment II Growth Curves.



This slow growth was evident from the first week, but during the last two weeks there was slightly better growth. Most of the chicks on this diet feathered poorly, and were very much given to cannibalistic habits. Table XIV illustrates further that the fish meal group was significantly poorer than all the others.

The whale meat meal gave good growth for the entire period. The growth could not be considered quite normal, but this was probably due to very hot weather experienced during the time the chicks were in the experiment. The males on the whale meal supplement were significantly heavier than those on crayfish meal, meat meal, and meat and bone meal, while the females showed no superiority over those in the crayfish meal group, and were only just significantly heavier than the females on the two different kinds of meat supplement.

The growth of all chicks (male and female) on crayfish meal, meat meal and meat and bone meal was almost identical.

Chicks which died during the first two days of the experiment were disregarded; this procedure accounts for the differences in numbers shown in Table XIII. All groups showed some degree of paralysis, those with the two meat meal supplements and whale meat meal being the worst in this respect. Only one case of paralysis was observed among the chicks on fish meal.

The mortality was highest on the crayfish meal supplement. The chicks on the crayfish meal diet drank much more water than the others, and the droppings were much softer, amounting in some cases to a mild diarrhoea. This physiological disturbance was probably due to the higher salt content of the crayfish meal.

The poor growth of chicks on fish meal could not be explained at this stage. The lack of some essential amino acid, or of some growth promoting factor of the B complex was suspected. With this in view the next experiment was planned.

Experiment III.

This experiment was designed to explain the poor results obtained with fish meal in the previous experiment. Lots I and II were used as controls, I containing fish meal alone and II meat and bone meal. In lot III, the fish meal was supplemented with 3 per cent. dried brewers' yeast. If the fish meal was deficient in a growth promoting factor of the B complex, the yeast ought to supply it, and thus improve growth.

In lot IV, one half of the protein supplied by fish meal was replaced by the quivalent amount of protein from casein, the latter being low in cystine.

In lot V, one half of the protein was replaced by peanut meal.

To test any supplementary action between fish meal and meat meal, an extra lot VI was included, each supplying half the protein supplement.

The actual rations fed are given in Table XVI.

TABLE XVI.

Rations.

	Lot I.	Lot II.	Lot III.	Lot IV.	Lot V.	Lot VI.
Wheat bran.......	15·0	15·0	15·0	15·0	15·0	15·0
Pollard...........	10·0	10·0	10·0	10·0	10·0	10·0
Lucerne meal.....	5·0	5·0	5·0	5·0	5·0	5·0
Yellow maize meal.	57·5	51·5	56·5	58·5	55·0	56·0
Fish meal.........	10·0	—	8·0	5·0	5·0	5·0
Meat and bone meal	—	17·0	—	—	—	7·5
Casein............	—	—	—	4·0	—	—
Peanut meal......	—	—	—	—	7·5	—
Brewers' yeast....	—	—	3·0	—	—	—
Bonemeal.........	1·0	—	1·0	1·0	1·0	1·0
Limestone flour...	1·0	1·0	1·0	1·0	1·0	1·0
Salt..............	·5	·5	·5	·5	·5	·5
Per cent. Protein..	18·4	18·0	17·8	17·5	17·5	17·3

TABLE XVII.

Average Weekly Weights.

	Lot I.	Lot II.	Lot III.	Lot IV.	Lot V.	Lot VI.
Day old..........	35·7	35·6	36·3	35·5	36·4	36·6
1 week...........	46·9	47·1	46·9	50·4	47·9	44·9
2 weeks..........	63·5	69·1	68·8	73·8	66·7	62·6
3 weeks..........	81·4	98·7	97·9	102·5	90·3	85·7
4 weeks..........	106·5	130·9	132·0	139·0	120·5	114·6
5 weeks..........	139·6	173·9	190·3	188·9	161·3	153·0
6 weeks..........	171·7	224·7	254·5	243·8	208·1	198·1
7 weeks..........	218·6	286·4	323·3	315·0	272·7	254·3
8 weeks..........	264·2	347·6	414·9	391·2	345·5	313·8
Males............	271·9	372·9	409·6	410·0	360·3	332·0
Females..........	259·8	324·4	425·5	371·3	331·5	305·2

TABLE XIX.

Health and Mortality.

Lot.	No. of chicks started.	Paralysis.	Mortality.	Percentage Paralysis.	Percentage Mortality.
I......................	36	2	7	5·6	19·4
II.....................	36	5	9	13·9	25·0
III....................	36	—	5	—	13·9
IV.....................	36	24	3	66·7	8·3
V......................	36	11	3	30·6	8·3
VI.....................	37	3	3	8·1	8·1

TABLE XVIII.

Food Consumption and Growth.

Age.	Lot I.			Lot II.			Lot III.			Lot IV.			Lot V.			Lot VI.		
	Total food consumed. Grams	Grams food per gram grain.	Grams gain per gram protein.	Total food consumed. Grams	Grams food per gram gain.	Grams gain per gram protein.	Total food consumed. Grams	Grams food per gram gain.	Grams gain per gram protein.	Total food consumed. Grams	Grams food per gram gain.	Grams gain per gram protein.	Total food consumed. Grams	Grams food per gram gain.	Grams gain per gram protein.	Total food consumed. Grams	Grams food per gram gain.	Grams gain per gram protein.
1 week..........	1,055	3·05	1·78	1,010	2·93	1·89	943	2·88	1·94	1,180	2·33	2·37	1,060	2·64	2·17	895	3·17	1·83
2 weeks..........	1,685	3·27	1·66	1,780	2·41	2·33	1,860	2·73	2·06	2,080	2·56	2·06	2,000	3·04	1·88	1,980	3·29	1·75
3 weeks..........	2,055	3·70	1·47	2,390	2·89	1·92	2,905	3·22	1·74	2,710	2·87	1·93	2,630	3·16	1·81	2,890	3·68	1·57
4 weeks..........	2,650	3·41	1·58	3,315	3·67	1·51	3,670	3·47	1·61	3,715	3·05	1·81	3,525	3·34	1·71	3,485	3·54	1·63
5 weeks..........	3,940	4·07	1·34	4,335	3·60	1·54	5,900	3·27	1·73	5,325	3·26	1·69	5,130	3·59	1·59	5,125	3·93	1·47
6 weeks..........	4,372	4·65	1·17	5,285	3·98	1·39	7,305	3·67	1·53	6,382	3·52	1·57	5,895	3·76	1·52	6,000	3·90	1·48
7 weeks..........	5,233	3·84	1·41	6,065	3·63	1·53	8,075	3·78	1·49	7,713	3·28	1·68	7,170	3·40	1·68	7,235	3·79	1·53
8 weeks..........	6,125	4·63	1·17	7,510	4·55	1·22	11,055	3·90	1·44	9,060	3·60	1·53	8,675	3·61	1·58	8,385	4·15	1·39
	27,115	3·996	1·360	31,690	3·658	1·519	41,713	3·555	1·580	38,165	3·238	1·702	36,075	3·453	1·655	35,995	3·819	1·510

TABLE XX.

Differences in Mean Weight at Eight Weeks of Age.

Lots.	Males.			Females.			Unweighted means of males and females.		
	Difference.	T.	P.	Difference.	T.	P.	Difference.	T.	P.
1 & 2	100·7	3 6321	< ·01	64·6	2·5394	·01	82·65	4·3985	< ·01
1 & 3	137·7	6·1024	< ·01	165·7	7 0488	< ·01	151·70	8·1410	< ·01
1 & 4	138·1	5·2858	< ·01	111·5	4·5520	< 01	124·80	6·9703	< ·01
1 & 5	88·4	3·3413	< ·01	71·7	2·9711	< ·01	80·05	4·4709	< ·01
1 & 6	60·1	2·2386	< ·02	45·4	1·8813	> ·05	52·75	2·9235	< ·01
2 & 3	37·0	1·3035	> ·05	101·0	4·1431	< ·01	69·05	3·6896	< ·01
2 & 4	37·4	1·4628	> ·05	46·9	1·8499	> 05	42·15	2·3431	·03
2 & 5	12·3	·4753	> ·05	7·1	·2840	> ·05	2·60	·1445	> ·05
2 & 6	40·6	1·5691	> 05	19·2	·7678	> ·05	29·90	1·6495	> ·05
3 & 4	·4	·015	> ·05	54·2	2 3056	·03	26·90	1·5089	> ·05
3 & 5	49·3	1·839	> 05	94 0	4·0640	< ·01	71·65	4·0190	< ·01
3 & 6	77·6	2·8217	< ·01	120·3	5·2010	< ·01	98·95	5·5072	< ·01
4 & 5	49·7	2·0584	·05	39·8	1·6493	> ·05	44·75	2·6225	·01
4 & 6	78·0	3·1738	< ·01	66·1	2 7391	·01	72·05	4·1866	< ·01
5 & 6	28·3	1·532	> ·05	26·3	1·1067	> ·05	27·3	1·5863	> ·05

TABLE XXI.

Mean Weights and Standard Errors of Chicks at Eight Weeks.

Lot.	Males.	Females.	Unweighted Males and Females.	No. of Males.	No. of Females.	Total.
I....	271·9 ± 20·00	259·8 ± 17·32	265·8 ± 13·23	12	16	28
II....	372·6 ± 19·22	324·4 ± 18·52	348·5 ± 13·34	13	14	27
III....	409·6 ± 20·89	425·5 ± 15·90	417·6 ± 13·13	11	19	30
IV....	410·0 ± 16·81	371·3 ± 17 32	390·7 ± 12·07	17	16	33
V....	360·3 ± 17·32	331·5 ± 16·81	345·9 ± 12·07	16	17	33
VI....	332·0 ± 17·89	305·0 ± 16·81	318·6 ± 12·27	15	17	32

The weekly average live weights of all chicks, and the average of male and female chicks at eight weeks of age are presented in Table XVIII. The growth curves are presented in Fig. 3. The total food consumption, the food requirement per unit gain, and the gain per unit protein ingested, are presented in Table XVIII.

Fig. III.

Experiment III. Growth Curves.

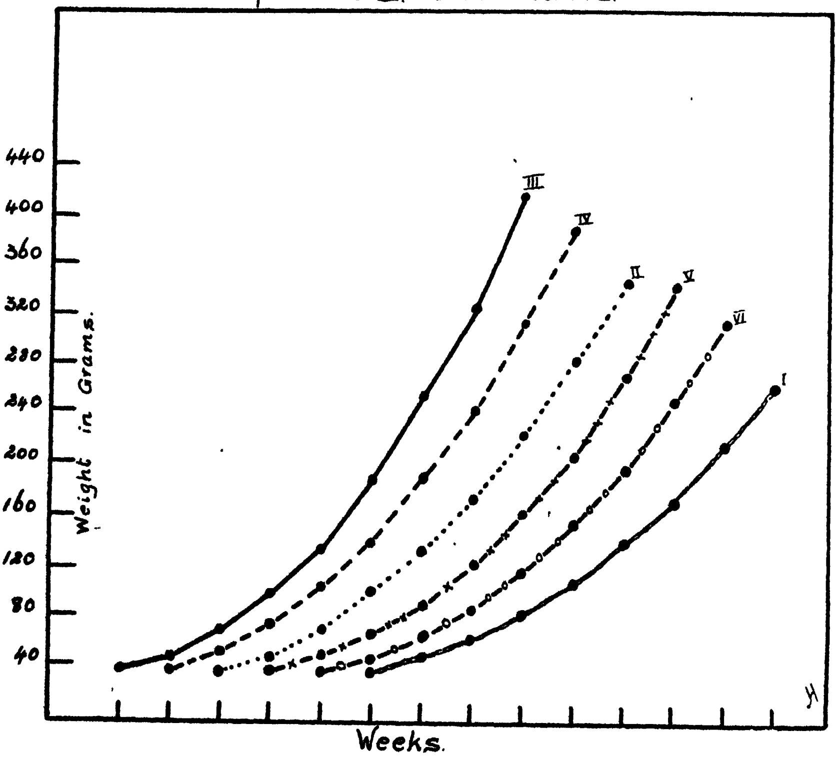


Table XIX gives the health and mortality records of chicks in the different groups.

The differences in mean weight at eight weeks of age, together with Fisher's T values and approximate probabilities are presented in Table XX.

Discussion.—The poor results with fish meal as a protein supplement for the growth of chicks was duplicated in this experiment. The slightly better growth was probably due to the fact that this experiment took place during autumn and early winter.

The most striking fact was the enhanced growth of chicks on fish meal, supplemented with dried brewers' yeast (Lot III). With casein as a supplement, the chicks grew very well for the first three weeks, but then showed a decline in growth and a high incidence of paralysis. The casein group showed better food utilization and greater gains per unit protein ingested. It would thus appear that the addition of casein improved growth, by supplying some factor essential for growth. The yeast supplement increased growth, but the biological value according to Osborne, Mendel and Ferry remained nearly eight per cent. lower than where casein was utilized. The biological value of fish meal is thus lower than that of fish meal supplemented by casein. At the same time, fish meal apparently lacks at least two factors supplied by dried brewers' yeast. The first is a growth promoting factor also contained in casein, and the second is a factor which prevents paralysis. Casein is also deficient in this anti-paralysis factor.

The meat and bone meal used in this experiment also proved to be inferior to fish meal supplemented with yeast or casein, i.e. lots 3 and 4 grew significantly better than lot 2. Thus meat and bone meal supplemented fish meal to some extent, but growth was significantly poorer than in group 2 where all the animal protein was supplied by meat and bone meal.

The biological values obtained for lots II and VI are, however, essentially the same. It would thus appear that the meat and bone meal used was also deficient in this growth promoting factor, as the quantity used to supplement the fish meal in lot VI did not supply enough for optimum growth. Another explanation would, of course, involve a lack in each of the same essential amino acids; this seems hardly possible.

Peanut meal supplemented fish meal to about the same extent as meat meal. As in the case of lot III, but not to so marked a degree, there was a deficiency of the anti-paralysis factor. This complicates the interpretation as far as growth is concerned, as the paralysis interferes rather seriously with feeding, and the paralysis factor itself might be growth promoting.

Summary.

1. Significant differences in the growth promoting ability of meat meals was demonstrated.

2. One sample of fish meal tested gave extremely poor growth.

3. It was shown that fish meal lacks at least two factors:—a growth promoting factor and a factor which prevents paralysis.

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